TECHNICAL REPORT
FD-36

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Department of Nutrition and Food Science
Cambridge, Massachusetts

Contract No. DA 19-129-AMC-254 (N)

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U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
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FOREWORD

Dehydrated foods are particularly susceptible to autoxidation; oxidative deterioration of the lipids and secondary reactions proceeding therefrom have serious consequences on quality. In order to improve the stability of dehydrated foods for military use, it is important to understand both stabilizing and destabilizing subsystems. Amino acids have been known to affect the course of autoxidation of food lipids, but have not been studied systematically in dehydrated systems.

The work covered in this report, performed in the Division of Sponsored Research, Massachusetts Institute of Technology under Contract Number DA19-129-AMC-254(N) (April 1964-April 1965) represents the first phase of an investigation into the determination of the antioxidant and/or prooxidant effects of various amino acids in model systems simulating dehydrated foods and of the nature of the observed antioxidant activity with a view toward the elucidation of their mechanisms. The investigator was Dr. M. Karel; his collaborator was Dr. S. Tannenbaum.

The U. S. Army Natick Laboratories Project Officer was A. S. Henick, and the alternate Project Officer was S. J. Bishov both of Food Chemistry Branch, Food Division.

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SUMMARY

Oxidation of methyl linoleate was studied in a freeze-dried model system based on microcrystalline cellulose, in the presence and in the absence of amino carboxylic compounds added in concentrations ranging from 10^{-3} to 4×10^{-2} moles per mole of linoleate. It was observed that several amino carboxylic compounds had substantial antioxidant activity. Compounds for which such activity was observed included: histidine, cysteine, alanine, lysine, beta amino butyric acid, gamma amino butyric acid, and epsilon amino caproic acid; whereas no antioxidant activity was observed with methionine, arginine, phenylalanine and isoleucine.

The nature of the antioxidant activity of the amino compounds was found to be different from that observed in the identical system with a phenolic antioxidant, since the main effect of the amino compound was to prolong the induction period, but the phenolic compound prolonged the induction period, and in addition reduced the rate of oxidation throughout the course of oxidation.

Work was undertaken on the separation and characterization of reaction products from model systems containing linoleate and amino compounds. Reaction products in the model system oxidized in the presence of linoleate and histidine were studied using radioactive tracer compounds. Reaction products derived from histidine were located through the use of ring labelled histidine (C^{14}) and those derived from linoleate through the use of uniformly labelled methyl linoleate (C^{14}). Fractions of the

reaction products were obtained by chromatographic and electrophoretic techniques. The fractions were characterized on the basis of the source of radioactivity, relative mobility in different solvent systems and presence of specific functional groups as determined with suitable reagents.

Reaction products from the model system containing linoleate and methionine were studied using C^{14} labelled methionine. Methionine sulfoxide was found to be a major reaction product, and it was formed only in the presence of oxidizing linoleate. No methionine sulfone was found under these conditions.

I. INTRODUCTION

Amino acids and proteins have been known to affect the course of autoxidation of lipids in foods, but systematic studies of their prooxidative and antioxidative effects have been few. In 1961, Marcuse reported on a study of the antioxidative effects of several amino acids added to aqueous solutions of linoleate at pH 7.5. He experienced the usual difficulties in reproducing in repeated experiments the magnitudes of the antioxidative effects, but was able to conclude the following:

1. Several amino acids, including histidine, alanine, methionine and lysine reduced the oxygen absorption by linoleate by as much as 50 to 80%.
2. Each amino acid had an optimum concentration for the antioxidant activity, and at high concentrations showed an activity inversion, becoming prooxidative rather than antioxidative in its action.
3. The antioxidative activity depended on pH, presence of other antioxidants or synergists, and on the state of oxidation of the linoleate.

More recently several studies have been reported on the prooxidative effects of amino acids, especially that of histidine, on linoleate in aqueous emulsions. The same factors which affected the antioxidant activity of the amino acid were shown to be involved in the prooxidant activity.

Among the food products particularly susceptible to autoxidation are dehydrated foods, in which the oxidation of lipids may have serious consequences on quality. Oxidation causes deterioration of lipids, and secondary reactions between lipid oxidation products and proteins lead to

browning, loss of protein quality and impaired organoleptic quality. The present study was undertaken in order to explore in a model system designed to approximate conditions in freeze-dehydrated foods, the interaction between amino acids and lipid oxidation. The study has the following objectives:

1. To determine the antioxidant and/or prooxidant effects of various amino carboxylic compounds present in model systems containing methyl linoleate and simulating dehydrated foods.
2. To study the nature of the observed antioxidant activity of the amino carboxylic compounds with a view towards the elucidation of the mechanism of such effects.

II. OXIDATION EXPERIMENTS

A. EXPERIMENTAL

1. Materials

a. Methyl Linoleate. Purified methyl linoleate was obtained from Mann Research Laboratories, Inc. The following lots were received for use in our experiments: #L 2235, #M 1298, #M 2081, #M 2525, #M 2410, #M 2066. The methyl linoleate was further purified by urea adduct formation in methanol, and thorough washing with urea saturated methanol. The adduct was then dissolved in water and solvent extracted with benzene to remove the methyl ester. As a further precaution in later runs the benzene layer was washed with 0.1 M citric acid and

then rewashed with water. The benzene was then evaporated under vacuum and the methyl linoleate was vacuum distilled. Only the center cut of the distillate was used for subsequent oxidation studies. Greatest care was exercised to avoid oxidation and/or contamination during the purification procedures.

The effectiveness of the purification is indicated by typical properties of the ester before and after purification as shown in Table 1.

TABLE 1. Effectiveness of purification of methyl linoleate.

	Typical properties	
	Before purification	After purification
Diene conjugation, %	0.2 to 2.0	0.001 to 0.1
Iron, μg/g	0 to 0.4	0 to 0.2
Copper, μg/g	0.1 to 4.0	Undetectable
Induction period, hr	0 to 5	5 to 30

In addition the purification procedure was checked by thin layer chromatography to assure absence of peroxides and other contaminants detectable by this method. The method used was essentially the same as that reported by Privett (1962). Further assurance of purity was obtained by gas chromatographic analysis of the ester both before and after purification to assure the absence of contamination by methyl esters other than linoleate.

The gas chromatographic analysis was carried out on one to two microliters of a one percent solution in n-hexane of the methyl ester to be analyzed; standard mixtures of methyl esters of fatty acids were used for calibration. An F & M Model 1609 flame ionization gas chromatograph was used under the following operating conditions:

column type -- 8 ft, 10% LAC 728 on 60-80 W. 609

column temperature -- 190°C

carrier gas flow rate -- 60 ml/min

sensitivity -- 100 x 16 or 100 x 8

Methyl linoleate-U-C^{14} (Applied Science Laboratories) was used in the analysis of the oxidized model system.

b. Carbohydrate matrix. Microcrystalline cellulose (Avicel, American Viscose Co.) was used without further purification. Previous studies in the department indicated that the material had the most reproducible physical and chemical properties of all materials under consideration, especially with respect to low heavy metal content.

c. Amino carboxylic compounds. All of the amino carboxylic compounds to be used in the present study, with the exception of radioactive compounds used for tracer analyses, were obtained from CALBIOCHEM, Los Angeles, California. The highest grade available was obtained, and in most cases (grade A) had guaranteed chromatographic purity. All compounds having no guarantee of purity (grade C) were tested for homogeneity

using the chromatographic procedures of Fahmy et al. (1961). Methionine methyl labeled C^{14}-methionine was obtained through the courtesy of Dr. Mateles of our department. The amino acid was originally obtained from Atomic Accessories Division of Baird Atomic (Valley Stream, New York). Labeled L-histidine (2-ring C^{14}) was obtained from Nuclear Chicago.

The amino carboxylic compounds used in this study are listed below:

Compound	Source	Purity (Grade)
DL-Alanine	Calbiochem	A
L-Arginine	"	A
DL-β-Amino n-Butyric Acid	"	C
γ-Amino Butyric Acid	"	A
ϵ-Amino-n-Caproic Acid	"	C
L-Cysteine Hydrochloride Hydrate	"	A
L-Histidine	"	A
L-Isoleucine	"	A
DL-Lysine·HCl	"	A
DL-Methionine	"	A
DL-Phenylalanine	"	A
Methionine-methyl labeled C^{14}-methionine	Atomic Accessories (Division of Baird Atomic, Valley Stream, N.Y.)	
L-Histidine (ring-2-C^{14})	Nuclear Chicago	

d. Water. All the water used in this study was deionized and redistilled shortly before use.

2. Procedures

a. Preparation of the model system. The steps followed in the preparation of the model system are shown in Table 2.

TABLE 2. Model system based on Avicel.

Formula:	Methyl linoleate, purified	1 g
	Avicel	6 g
	Water, redistilled	30 g

Preparation:

1. Components mixed in Omni-mixer for 5 minutes, under nitrogen blanket.
2. Viscous suspension extruded into reaction flasks.
3. Flasks immersed in liquid nitrogen.
4. Flask contents freeze-dried at: 100 microns pressure ambient platen temperature for 24 to 36 hours.
5. Vacuum in drier broken with dry nitrogen.

Reaction flasks contained a known amount of the reaction mixture, usually calculated to give approximately 200 mg of methyl linoleate per flask. The freeze drier used for the dehydration was described previously (Goldblith et al., 1963).

b. Oxidation. The oxidation of model systems was followed by the following methods. The Warburg reaction flasks were attached to Warburg manometers and the oxidation conducted

under dry air at 37°C, in a Warburg bath. Standard manometric procedures were followed for calibration and calculation of results (Umbreit, 1964). The atmosphere over the reaction mixture was checked for possible carbon dioxide production using gas chromatographic analysis, and no carbon dioxide was observed.

In order to eliminate the possibility of oxygen depletion, the reaction flasks were flushed at suitable intervals with dehumidified air.

In addition to the manometric determination of oxygen absorbed similar reaction flasks were placed in a bath at 37°C, under dry air, and the samples used for U.V. spectrophotometric analysis of diene conjugation at 233 mμ (Privett, 1962). Extraction of the lipid phase for the spectrophotometric analysis was done with methanol. The extraction procedure was previously checked and found to be both efficient, and sufficiently mild to assure that no oxidation takes place during the extraction.

3. Presentation of Results

The results of oxidation experiments are presented with the degree of oxidation reported as moles of oxygen absorbed per mole of linoleate originally present.

The autocatalyzed oxidation of unsaturated fatty acids, and of their esters, is known to have at least two stages separable on the basis of kinetic considerations (Lundberg, 1961). In the initial stage, during which the oxidation is

catalyzed by hydroperoxides decomposing into radicals by a scheme involving monomolecular decomposition, the rate of oxygen absorption may be expressed by equation 1.

(Eq. 1) $\frac{dy}{dt} = ky^{1/2}$

where:

y = oxygen absorbed in moles per mole of linoleate

t = time in hours

k = a constant, including many of the important factors held constant in a given experiment

While this equation is only approximate, it does hold well for oxidation of pure substrates, including methyl linoleate (Bateman, 1954; Kern and Dulog, 1959).

On integration of equation 1, it becomes apparent that a plot of $y^{1/2}$ vs t should result in a straight line provided monomolecular decomposition of hydroperoxides is responsible for initiation of the autocatalytic chain. Changes in slope of the straight lines due to addition of amino acids indicate antioxidant or prooxidant effects operative in this period of oxidation.

Calculations in accordance with equation 1 have been made and results presented for the cases where an effect in the monomolecular period was demonstrable. In addition, the results of all oxidation experiments in the early stages of oxidation are presented simply as oxygen absorbed vs. time (y vs. t).

In the subsequent rapid phase of oxidation, the reaction is catalyzed by bimolecular decomposition of hydroperoxides; in addition, depletion of the unoxidized substrate becomes sufficiently significant to affect the rate of oxidation. Under these conditions, an approximate equation, considered to give the dependence of oxygen absorption rate on the amount of oxygen absorbed, is equation 2.

(Eq. 2)

$$\frac{dy}{dt} = (K)(y)(RH)$$

where:

K = the bimolecular phase reaction rate constant, which includes many factors held constant in an experiment

RH = the concentration of unoxidized linoleate (moles per mole of linoleate originally present)

The other symbols have been defined previously.

If it is assumed that one molecule of oxygen is absorbed per molecule of linoleate (certainly a reasonable assumption for the states of oxidation discussed here), then the relation between oxygen absorbed and unoxidized linoleate is given by equation 3.

(Eq. 3)

$$RH = (1-y)$$

Equation 2 may then be rewritten as follows: (equation 4)

(Eq. 4)

$$\frac{dy}{dt} = Ky(1-y)$$

On integration it becomes obvious that a plot of ln [y/(1-y)] vs. t should give a straight line. This method of plotting allows graphical representation of the beginning of the bimolecular decomposition period, and changes in slopes of the straight lines indicate the existence of inhibitory or catalytic effects in this period. The data in this report, therefore, are presented as plots of [y/(1-y)] on semilogarithmic coordinates against time. It should be realized that at low levels of oxidation the correction (1-y) is not very significant and the plot is essentially one of oxygen absorbed vs. time. As the oxidation degree increases, the corrected value becomes significantly different from y. For instance, when oxygen absorbed is 0.050 moles per mole, the corrected value is 0.053; when y has risen to 0.100, the corrected value is 0.111; at a value of y equal to 0.200, the corrected value is 0.250. (The experiments reported here are terminated at oxidation levels of 0.135 to 0.20 moles of oxygen per mole of linoleate.)

B. RESULTS AND DISCUSSION

1. Results

The effect of addition of amino carboxylic compounds on rate of oxidation in the model system was studied in ten separate runs in which oxygen absorption was followed manometrically and by measurement of increase in diene conjugation.

The results of these runs are presented as follows:

Run I: Control, histidine (10^{-2} and 10^{-3} M/M)* in Table 3 and Figures 1, 2, and 3.

Run II: Control, methionine (4×10^{-2} and 10^{-3} M/M) in Table 4 and Figures 4 and 5.

Run IV: Control and histidine (10^{-3} and 10^{-4} M/M) in Table 5 and Figures 6 and 7.

Run V: Control, histidine (10^{-3} M/M) and cysteine (2×10^{-3} M/M) in Table 6 and Figures 8, 9 and 10.

Run VI: Control, histidine (10^{-3} M/M) and propyl gallate (10^{-3} M/M) in Table 7 and Figures 11, 12 and 13.

Run VII: Control, lysine (10^{-3} M/M) and β-amino-n-butyric acid (10^{-3} M/M) in Table 8 and Figures 14, 15 and 16.

Run VIII: Control, cysteine (10^{-3} M/M) and ϵ-amino-n-caproic acid (10^{-3} M/M) in Table 9 and Figures 17 and 18.

Run IX: Control, arginine (10^{-3} M/M) and phenylalanine (10^{-3} M/M) in Table 10 and Figures 19 and 20.

Run X: Control, alanine (10^{-3} M/M) and γ-amino-n-butyric acid (10^{-3} M/M) in Table 11 and Figures 21 and 22.

Run XI: Control, arginine (10^{-3} M/M) and isoleucine (10^{-3} M/M) in Table 12 and Figures 23 and 24.

*The concentrations of added amino compounds are expressed in moles of additive per mole of methyl linoleate.

In addition oxygen absorption was followed in detail in the preparative run for samples to be used in analytical studies B and C. The results of this preparative run, in as far as oxygen absorption is concerned are presented in Figure 25. The run has been labelled Run Z. The concentration of histidine in this run was 9×10^{-3} M/M.

In the subsequent discussion, these results will be discussed separately for each treatment. Thus, reference may be made to several of the runs for the treatments which were repeated in different runs.

It is obvious from the results, that considerable difficulty was encountered in reproducing induction periods for the controls. In replicate runs, furthermore, variations were observed in the magnitude of the antioxidant activity of the amino acids. These difficulties, were similar in nature to those experienced by Marcuse (1961) in a much simpler system, consisting of a solution without the cellulosic supports, which were used in the present study.

In comparing the effect of different amino acids, therefore, it is necessary to relate this effect to the behavior of the control samples used in the same run under identical conditions with the treated samples. In order to compare these effects a presentation is made in Table 13 of the following values for each run:

1. The time required by the control to reach an oxidation level of 10 mmoles of oxygen/moles linoleate; and

the time required by the treated sample in the same run, under identical conditions.

2. Relative rates of oxygen absorption for each treatment with respect to its own control. These refer to the amount of oxygen absorbed by the treated sample, divided by the amount absorbed by the control, at a time when the control oxidation level is 10 mmoles/mole. In addition to our experimental values, the relative absorption values reported by Marcuse (1961) are presented for comparison.

The comparison with the data of Marcuse, is interesting, but it should be recognized that the conditions of his experiments were entirely different from ours. The reason for presenting them is to point out some striking similarities, in spite of different experimental conditions.

2. Discussion

a. L-histidine (Runs: I, IV, V, VI and Z). As reported previously, histidine in concentrations of 10^{-3} moles per mole of linoleate, shows an antioxidant effect in the induction period and in the early stages of oxidation. This inhibitory effect vanishes as the oxidation progresses to the bimolecular stage of oxidation.

The time necessary to reach a level of oxidation of 10 mmoles/mole, as compared with the control was increased in every case (Table 13). The relative oxygen absorption ranged from 0.237 to 0.685, as compared with values of approximately 0.68 reported by Marcuse (1961).

A detailed inspection of the results reveals that the antioxidant activity is limited to the induction period and possibly also to part of the monomolecular decomposition period. The conclusion that the effect of histidine does not extend to the bimolecular period of oxidation may be demonstrated by inspection of the data for the bimolecular period of oxidation which are presented in Figures 3, 7, 10 and 13. It is evident that addition of histidine displaces the straight lines along the time axis, confirming the previously mentioned delay in the onset of bimolecular decomposition. The straight lines themselves, however, have approximately the same slope, even in the case of Run I (Figure 3) in which the delay is almost 40 hours. This may be compared with the behavior of a typical phenolic antioxidant (Figure 13) which in addition to giving some delay in onset of bimolecular decomposition also lowers significantly the rate of oxidation in this phase of oxidation, as evidenced by the substantially reduced slope of the straight line.

The results obtained with other concentrations of histidine (10^{-4} moles/mole, and 10^{-2} moles/mole) show similar qualitative pattern but are less effective than the concentration of 10^{-3} moles/mole.

The delay in onset of rapid oxidation effected by histidine was most pronounced in Run I, in which the control had an induction period of 35 hrs, and was less in Runs V, VI and IV

in which the induction period was 16 to 21 hrs. However, in Run Z, histidine resulted in substantial inhibition of oxidation in spite of the very short control induction time of 7.4 hrs.

Analysis of data from the monomolecular period of oxidation (Figures 2, 9 and 12) indicated that some of the antioxidant activity of histidine may be still observed in this period; however, the main effect appears to occur in the induction period.

b. DL-Methionine. Results of experiments using this amino acid (4×10^{-2} and 10^{-3} moles/mole of linoleate) indicate that under conditions investigated here the amino acid has little if any antioxidant activity.

There is an indication of some prooxidant activity, as indicated by the shortening of the induction time from 37.5 to 33.5 hours, and a relative oxygen absorption of 1.36. The prooxidant activity, if real, does not seem to extend to the bimolecular decomposition period (Figure 5) and is not apparent in the diene conjugation increase (Table 4). The only conclusion possible, therefore, is that the amino acid has relatively little significant activity in either direction.

c. L-Cysteine. The effect of addition of cysteine was studied in Runs V and VI. In Run V, due to technical difficulties, the linoleate content of the cysteine-containing model was reduced, and the cysteine concentration was 2×10^{-3} moles/mole linoleate. The amino acid showed an antioxidant effect

comparable in magnitude with that of histidine. The relative absorption of oxygen was 0.605, and the time to reach 10 mmoles of oxygen/mole was increased with respect to the control (Table 13). All of the inhibitory activity seems to be due to extension of the induction period, since the slopes of the oxygen absorption curves in both the monomolecular period (Figure 9) and the bimolecular period (Figure 10) were essentially equal to those for the control.

The experiment was repeated in Run VIII, in which the cysteine was added in concentration of 10^{-3} M/M. The addition of the amino acid increased the time required to reach an oxidation level of 1% by 56%. The relative oxygen absorption was 0.4 (Table 13).

d. DL-Lysine. Addition of lysine was studied in Run VII. The amino acid was found to possess antioxidant activity, almost doubling the time required to oxidize to a level of 1%, and giving a relative oxygen absorption of 0.184. Little of the effect persisted in the rapid bimolecular phase (Figure 16), but there is an indication that the rate of oxidation is lowered in the monomolecular phase (Figure 15).

e. ϵ-amino-n-caproic acid. The addition of this compound in concentration of 10^{-3} M/M was studied in Run VIII. A substantial increase in induction time was observed. The control sample reached a level of oxidation of 1% in 16 hrs., the treated sample in 39 hrs. The relative oxygen absorption was 0.180 (Table 13). As in the case of lysine,

the inhibition appears to be limited to the induction phase and early stages of oxidation. In the bimolecular phase, the rates of oxidation for the control and the treated samples are approximately equal (Figure 18).

f. β-amino-n-butyric acid. The addition of this compound in a concentration of 10^{-3} M/M was studied in Run VII. Substantial inhibitory activity was observed. The time to reach a level of oxidation of 10 mmoles of oxygen per mole of linoleate was tripled, compared with the control, and the relative oxygen absorption was 0.132 (Table 13). The rates of oxidation in the rapid bimolecular phase of oxidation were not affected (Figure 16), but the amino compound appeared to not only prolong the induction period but also to reduce the rates of oxidation in the monomolecular period (Figure 15).

A repeat run was attempted, but due to contamination of the linoleate by an unknown compound which produced an emulsion during the purification procedure, the results were not considered reliable, and no further conclusions could be drawn.

g. γ-amino-n-butyric acid. The addition of this compound was studied in Run X. It was added in a concentration of 10^{-3} M/M. Some inhibitory activity was observed, but it was substantially lower than with β-amino-n-butyric acid. The relative oxygen absorption was 0.74 (Table 13). The effect was limited to early stages of oxidation, since the slopes of

oxygen absorption curves in later stages are identical to those for the control (Figure 22).

h. DL-Alanine. The effect of alanine addition was studied in Run X. An inhibitory effect was observed, the time to reach one percent oxidation being increased by 30%, and the relative oxygen absorption being equal to 0.48 (Table 13). As in the case of other amino acids, the effect was limited to the induction period and early stages of oxidation (Figures 21 and 22).

i. L-Arginine, L-Isoleucine and DL-Phenylalanine. The addition of these amino acids was studied in Runs IX and XI. All of the amino acids showed prooxidant activity limited to initial stages of oxidation (Figures 19, 20, 23 and 24). The extent of the prooxidant activity is given by the following values of relative oxygen absorption (from Table 13):

Arginine: 1.2 and 1.72

Isoleucine: 1.3

Phenylalanine: 1.5

The results of Runs IX and XI are, however, complicated by the fact that in these runs the methyl linoleate obtained from the Mann Research Laboratories behaved in an unusual manner during purification. In the extraction of the ester into benzene (see Experimental), an emulsion formed at the water-benzene interface. This not only made the purification

more difficult, but indicates the presence of an unusual impurity, which may affect the activity of amino acids. In fact, some additional experiments performed after the completion of the present project indicate that the impurity may be at least partially responsible for the prooxidant activity of the three amino acids discussed here. For the present, therefore, the only conclusion that we can draw is that under experimental conditions investigated these amino acids may behave as prooxidants.

j. Propyl Gallate. It seemed of interest to determine the behavior of a phenolic antioxidant of proven activity in the model system used in the present investigation. Consequently, an experiment was conducted (Run VI) in which propyl gallate was added to the model system in concentration of 10^{-3} M/moles of linoleate, and in a manner identical to the addition of amino carboxylic compounds. Propyl gallate was chosen as the antioxidant because:

(1) It has a known effectiveness in inhibiting oxidation of vegetable oils in which linoleic acid is a major component.

(2) It has sufficient solubility in water to be added in a manner identical to the addition of the amino carboxylic compounds.

The antioxidant was found to be quite effective in our model system. The time to reach 1% oxidation level was increased

from 17.5 hrs for the control to 44.5 hrs for the treated sample. The relative oxygen absorption was 0.184 (Table 13). The antioxidative action of propyl gallate in our system shows significant differences from the behavior of amino acids in the same system. In particular, the phenolic antioxidant retains an inhibitory activity throughout the course of oxidation. It not only prolongs the induction period, and clearly depresses the rate of oxidation in the monomolecular phase of oxidation (Figure 12), but it also significantly reduces the rate in the bimolecular period of oxidation (Figure 13).

It should be pointed out, however, that in the very early stages of oxidation (less than 1% oxidation), some of the amino acids studied have an effectiveness comparable to that of propyl gallate. At control oxidation level of 10 mmoles of oxygen per mole of linoleate (1% oxidation), the propyl gallate gave a relative oxygen absorption of 0.184; under the same conditions lysine gave a value of 0.184, β-amino-n-butyric acid of 0.132, ϵ-amino-n-caproic acid of 0.180; and in Run I histidine gave a value of 0.237.

III. REACTION PRODUCTS OF AMINO ACIDS IN OXIDIZING LINOLEATE SYSTEMS

In order to predict the conditions under which amino acids will be effective inhibitors of oxidation and the structural requirements for an effective inhibitor, it is necessary to understand the mechanism by means of which these compounds inhibit oxidation. One useful approach in this respect is to determine the reaction product(s) of the inhibitor in the oxidizing system. Studies have been underway during the course of this investigation on the reaction products of histidine and methionine in the freeze-dried amino acid-methyl linoleate-cellulose system. These studies have been developed with the aid of histidine-2-(ring)-C^{14}, methionine-methyl-C^{14}, and methyl linoleate-U-C^{14}.

A. STUDIES WITH HISTIDINE-C^{14}

1. Experimental

In order to obtain as high a concentration as possible of any products of histidine that might be formed in the course of the reaction, the model system has been oxidized to a relatively high level in most cases, namely 0.2 moles O_2/mole of linoleate. A limited amount of work has also been done at an oxidation level of 0.075 moles O_2/mole of linoleate. The work with histidine has been done at concentrations of 10^{-3} and 9×10^{-3} mole/histidine/mole linoleate.

A summary of the various systems explored indicating the concentration of histidine and the level of oxidation is given in Table 14.

A typical freeze-dried system was prepared in the usual manner and consisted of 2.5 grams methyl linoleate, 15 grams microcrystalline cellulose, and 1.28 milligrams (31 μc) L-histidine-2-(ring)-C^{14} (10^{-3} M/M methyl linoleate). The progress of oxidation was followed manometrically. A typical oxidation curve is shown in Figure 25 which presents the progress of oxidation during preparation of samples B and C.

As might be expected a priori, an extremely complex mixture of products was obtained. Analysis of the mixture was simplified somewhat since only products containing histidine were of interest in the present experiments.

Initially, the oxidized system was subjected to a procedure of gross fractionation as follows. A chromatographic column of dimensions suitable for the amount of material to be treated was packed (dry) with a 2 to 3 cm layer of chromatographic grade cellulose. The oxidized system was then transferred to the column with the aid of petroleum ether (b.p. 30-60°C) and an additional 2-3 cm of chromatographic grade cellulose was added. The column was then washed with four column volumes of petroleum ether and allowed to drain. This procedure has been demonstrated to remove 99+ % of unreacted methyl linoleate from the system (by gas-liquid

chromatography of the petroleum ether eluate). No detectable radioactivity from histidine was present in this eluate.

The column was then placed on a fraction collector and successively eluted with 4 column volumes of chloroform-methanol (4:1) up to 10 column volumes of chloroform-methanol with methanol concentration increasing to a ratio of 1:1, and up to 10 column volumes of methanol-water with water concentration increasing from 0 to 100%. Individual fractions of approximately 0.2 column volume were collected.

The distribution of radioactivity from histidine in these fractions indicated that the reaction products of histidine were distributed in 3 major peaks. The fractions under these peaks were pooled and designated as follows: the 4:1 chloroform-methanol eluate as Fraction I, the additional chloroform-methanol eluate as Fraction II, and the water-methanol eluate as Fraction III.

Fraction I is characterized mainly by the presence of some yellowish-brown material which fluoresces strongly under long-wave ultraviolet light (ca. 360 mμ) and of most of the materials giving positive peroxide tests. Fraction II also contains some fluorescent material, but is more polar than Fraction I. Fraction III contains most of the unreacted histidine and reaction products of histidine. A more detailed description of these fractions is given in the following section of the report.

In order to obtain more detailed information about the nature of the compounds in Fractions I, II, and III, these fractions were subjected to a detailed analysis in various chromatographic and electrophoretic systems. Reagents used to test for the presence of functional groups were as follows: ninhydrin (for α-amino group), diazotized sulfanilic acid (for imidazole ring), potassium iodide (for peroxides). Chromatograms were also observed under ultraviolet light, treated with iodine vapor, and scanned for radioactivity derived from histidine-C^{14}. In addition, Fraction III was refluxed with 6 N HCl and treated with cold and refluxing 55% HI to determine whether any change in the products would result.

2. Results and Discussion

The overall distribution of radioactivity from histidine (Study A) in the major fractions is given in Table 15. The details of the chromatographic analyses of these major fractions are given in Tables 16-18.

Only a small fraction of the radioactivity derived from histidine is observed in the non-polar fractions, and part of this radioactivity appears to be unchanged histidine. The bulk of the material is soluble in methanol and water. Since radioactive, ninhydrin-positive, and sulfanilic acid-positive components of Fraction III could be separated by paper electrophoresis in acidic buffers and by paper chromatography

in acidic solvents this fraction from Study A was subjected to two-dimensional chromatography and electrophoresis. The results of this separation are given in Table 19. The four sub-fractions obtained are all radioactive, ninhydrin-positive, and sulfanilic acid-positive. Of major interest is the fact that the bulk of radioactivity is not identical with unchanged histidine. Descending paper chromatography and thin layer chromatography on cellulose of Fraction III from studies B and C with the butanol-acetic acid-water system indicate that the same major reaction product of histidine is present in all systems.

The fluorescent materials in Fractions I and II appear to be derived largely from methyl linoleate. They do contain some carbon derived from histidine, but the results do not permit, as yet, a decision as to whether they are of importance to the antioxidant activity of histidine. The fluorescent materials are certainly of technological significance, since they represent a type of non-enzymatic browning in the absence of water.

Although the fluorescent materials have been chromatographed in a number of systems of varying polarity, a positive test for peroxide generally coincides with the fluorescence. The material does not migrate in an electrophoretic field but this does not necessarily indicate that it bears no charge: it may simply be due to insolubility in these systems. Any further discussion of the nature of structure of this material will have to await the accumulation of further evidence.

The information available on Fraction III from Study A indicates that less than one-third of the original histidine remains unchanged. Since separation has been achieved in general, in acidic, but not in basic systems, and since all of the fractions are ninhydrin-positive, it is possible to speculate that the changes take place on the imidazole ring.

A sufficient amount of Fraction III was isolated from Study C to enable a study of its reactivity with HCl and HI. Histidine is unreactive to refluxing HCl and cold HI, but a new product is formed from histidine with hot HI which is ninhydrin-positive and sulfanilic acid-negative. Fraction III is unchanged by hot HCl, but reacts both with hot and cold HI to give products not derived under similar conditions with histidine. Of major importance is the observation that under these conditions Fraction III appears to be partially reconverted to histidine. Since HI can catalyze a number of reactions (e.g. cleavage of epoxides, peroxides, ethers, etc.) it is premature to speculate on the nature of the change at this time.

B. STUDIES WITH METHYL LINOLEATE-U-C^{14} (WITH HISTIDINE AS AN ADDITIVE)

1. Experimental

Studies with radioactive methyl linoleate were initiated to determine whether the spectrum of oxidation products

changes in the presence of an amino acid inhibitor, and to determine if reaction products of the amino acid contained carbon derived from linoleate. The freeze-dried histidine model system was employed for all studies, and the conditions investigated were similar to those of the previous studies A, B and C (Table 14).

The oxidized system was treated for column chromatography as previously described for histidine with the exception that subsequent to elution with petroleum ether, the column was eluted successively with chloroform-methanol 4:1, 3:1, 2:1, 1:1 and water. The various fractions isolated in this manner were then examined with thin layer chromatography on silica gel using a variety of solvent systems (n-propanol-water, 2:1; dioxane-water, 3:1; chloroform; and 1,1,1-trichlorethane).

2. Results and Discussion

The results of the column chromatography of the linoleate-C^{14} systems is given in Table 20. Most of the radioactivity from the linoleate (approx 90%) is eluted with petroleum ether, and most of this radioactivity is in unchanged methyl linoleate. The bulk of the oxidation products derived from linoleate are eluted with chloroform-methanol 4:1 and these consist mainly of peroxides and unidentified fluorescent compounds.

The most significant result of these studies is that the distribution of the more water soluble oxidation products

does not appear to be a function of the presence of histidine. This result must be taken within the limitations of the method. As can be seen from Table 10, the unoxidized control had a significant amount of radioactive oxidation products. In almost all cases this material was chemically unmeasureable, although it behaved on thin layer chromatography in a manner identical to chemically detectable oxidation products from the oxidized samples. This is an indication that the specific activity of the impurities (oxidized linoleate) is higher than that of the methyl linoleate, a fact which makes it difficult to unequivocally interpret the results.

Paper chromatography of Fraction III indicates that there is either no or only a trace of radioactivity from linoleate associated with the major reaction product of histidine. This result probably eliminates the possibility of a molecule of histidine being combined with a large number of molecules of linoleate oxidation products. Since a typical experimental system contains in the range of 10^{-6} moles of histidine, it would be necessary to be able to detect in the order of 10^{-7} moles of linoleate to conclude its presence in a one molecule reaction product of histidine. Under the conditions of the present experiment, we cannot make this conclusion.

C. STUDIES WITH METHIONINE-METHYL-C^{14}

1. Experimental

Although methionine does not appear to act as an efficient inhibitor of linoleate oxidation in our system, previous information indicated that under certain conditions methionine does have antioxidant activity.

The freeze-dried model systems containing 10^{-3} moles of methionine/mole linoleate were prepared as previously described. A freeze-dried system containing methionine but not methyl linoleate was also investigated. The final specific activity of methionine in the system was 0.0177 μC/μM. After oxidation to a level of 0.2 mole O_2/mole linoleate the system was transferred to a chromatographic column as previously described and eluted successively with petroleum ether, chloroform, methanol and water. Over 95% of the radioactivity was eluted as a narrow band in the methanol fractions with the remainder tailing off into the initial water fractions. The methanol fraction containing the highest concentration of radioactivity was then concentrated in a stream of nitrogen and chromatographed on a thin layer of silica gel in propanol-water (2:1), and sec-butanol-acetic acid-water (4:1:1). Each of these systems clearly separates methionine, methionine sulfoxide, and methionine sulfone.

2. Results and Discussion

The results of the thin layer chromatography of the methionine systems is shown in Table 21. The only reaction product of methionine detectable with ninhydrin, iodine vapor and radioactivity was methionine sulfoxide. Methionine sulfone was not present, and methionine sulfoxide was formed only in the presence of oxidizing linoleate. Only a rough estimate of the amount of methionine converted to methionine sulfoxide can be made since only part of the column eluate was investigated. It would appear that the extent of conversion under the conditions of the investigation would be in the range of twenty percent.

Since methionine does not appear to act as an efficient inhibitor under the conditions investigated, it is possible that methionine is oxidized to the sulfoxide only when a significant concentration of peroxide has accumulated in the system. Thus, under some conditions, methionine might act as an antioxidant via peroxide decomposition. In view of the structure of the product, it would seem unlikely that methionine would act as a free radical acceptor.

The formation of methionine sulfoxide from methionine as a result of the oxidation of linoleate may be of major significance in terms of the nutritive value of proteins in rancid foods, and from this point alone is probably worthy of further investigation.

IV. REFERENCES

Bateman, L., Olefin oxidation, Quart. Revs. (London) 8, 147 (1954).

Coleman, J. E., J. W. Hampson, and D. H. Saunders, Autoxidation of fatty materials in emulsion. II. Factors affecting the histidine-catalyzed autoxidation of emulsified methyl linoleate, J. Amer. Oil Chem. Soc. 41, 347 (1964).

Fahmy, A. R., A. Niederwieser, G. Pataki and M. Brenner, Duennschicht-Chromatographie von Aminosaeuren auf Kieselgel. G. Eine Schnellmethode zur Trennung und zum qualitative Nachweis von 22 Aminosaeuren, Helv. Chim. Acta 44, 2022 (1961).

Goldblith, S. A., M. Karel and G. Lusk, The role of food science and technology in the freeze dehydration of foods, Food Technol. 17 (2,3), 139-144, 258-260, 263-264 (1963).

Karel, M. and S. Tannenbaum, The role of amino carboxylic compounds as oxidation inhibitors in dehydrated systems, Progress Reports #1 and #2, on Contract DA-19-129-AMC-254(N), Massachusetts Institute of Technology, Cambridge, Mass., 1964.

Kern, W., and L. Dulog, Ueber die Autoxydation ungesaettigter Verbindungen IX, Mitteilung. Der Anfangsverlauf der Autoxydation des Linolsaeuremethylesters, Makromol. Chemie 29 208 (1959).

Lundberg, W. O., ed., Autoxidation and Antioxidants, Vol. I, Interscience Publ., New York, N.Y., 1961.

Marcuse, R., Zur Wirkung von Aminosaeuren bei der Fett-Oxydation. II: Untersuch ungen mit Linoleat. Fette-Seifen-Anstrichmittel 63, 940 (1961).

Privett, O. S. and M. L. Blank, The initial stages of autoxidation. J. Amer. Oil Chem. Soc. 39, 465 (1962).

Umbreit, W. W., R. H. Burris and J. F. Stauffer, Manometric Techniques, Burgess Publ. Co., Minneapolis, Minn., 1964.

TABLE 3. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run I)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	2.24	0.70	0.69
44	44		
49		11.7	
58			4.64
70	183	25.4	
100		195	93

A = Control

B = 10^{-2} M histidine/M linoleate

C = 10^{-3} M histidine/M linoleate

TABLE 4. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run II)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0.0	2.63	2.96	2.11
11.0	4.2	3.91	3.74
30.3	12.7	7.76	5.83
51.0	41.0	22.8	--
66,5	--	--	29.8
77.0	58.7	54	--

A = Control

B = 4×10^{-2} M methionine/M linoleate

C = 10^{-3} M methionine/M linoleate

TABLE 5. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run IV)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	1.8	1.29	1.60
5.5	2.63	2.22	5.09
10.67	5.93	3.17	5.09
21.5	13.5	6.06	8.87
27	21.3	13.3	47.1
46	--	96.5	132.5

A = Control

B = 10^{-3} M histidine/M linoleate

C = 10^{-4} M histidine/M linoleate

TABLE 6. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

Time (hr)	(Run V) (M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	2.98	8.38	6.56
24	41.8	--	--

A = Control

B = 10^{-3} M histidine/M linoleate

C = 10^{-3} M cysteine/M linoleate

TABLE 7. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run VI)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	3.46	2.34	1.70
4	5.22	2.86	1.90
8	6.69	4.57	2.42
20	21.9	11.0	4.75
24	37.6	19.6	8.04

A = Control

B = 10^{-3} M histidine/M linoleate

C = 10^{-3} M propyl gallate/M linoleate

TABLE 8. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run VII)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	3.12	2.58	1.35
5	7.64	4.86	2.08
9	13.1	9.00	3.46
21	42.2	21.2	9.04
26.5	--	--	8.73
34.5	116.5	87.7	17.9

A = Control

B = 10^{-3} M lysine/M linoleate

C = 10^{-3} M β-amino-n-butyric acid/M linoleate

TABLE 9. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run VIII)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	6.74	1.82	1.51
5	8.01	2.14	2.06
15.5	9.63	4.22	4.26
21	19.6	--	--
34	--	5.60	3.34
45	51.6	12.1	--
58.5	--	39.4	12.2
78.5	--	--	25.1

A = Control

B = 10^{-3} M cysteine/M linoleate

C = 10^{-3} M ϵ-amino-n-caproic acid/M linoleate

TABLE 10. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run IX)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	2.22	2.25	1.94
8	4.44	--	--
20	11.0	--	--
22	20.8	29.4	18.1

A = Control

B = 10^{-3} M arginine/M linoleate

C = 10^{-3} M phenylalanine/M linoleate

TABLE 11. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run X)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	1.78	0.98	1.02
5	3.08	2.16	2.55
12	6.25	6.28	4.21
21	31.8	12.8	13.0
26	35.6	27.0	15.4

A = Control

B = 10^{-3} M γ-aminobutyric acid/M linoleate

C = 10^{-3} M alanine/M linoleate

TABLE 12. Oxygen absorption of methyl linoleate based on U.V. analysis of diene conjugation.

(Run XI)

Time (hr)	(M Oxygen/M Linoleate) x 10^3		
	A	B	C
0	2.14	2.76	2.64
6.5	3.77	3.60	4.21
12	5.56	6.50	6.32
20.5	15.5	16.0	14.2
25.5	26.8	17.4	24.2

A = Control

B = 10^{-3} M Isoleucine/M linoleate

C = 10^{-3} M Arginine/M linoleate

TABLE 13. Summary of the effect of amino acids on oxidation in early stages of oxidation.

Additive[1]	Run #	Time to reach oxidation level of 10 mM O_2/mole of linoleate (hr)		Oxygen absorption relative to control[2]	
		Control	Treated	Present study	Marcuse, 1961[3]
Histidine	I	33.5	76.25	0.237	0.68
Histidine	IV	20.5	24	0.685	0.68
Histidine	V	16.4	19.3	0.645	0.68
Histidine	VI	17.5	22	0.685	0.68
Histidine	Z[4]	7.4	13.2	0.474	0.68
Methionine	II	37.5	33.75	1.36	0.94
Cysteine	V[5]	16.4	19.8	0.605	>1
Cysteine	VIII	16	25	0.400	>1
Lysine	VII	10	19	0.184	0.81
α-amino-n-butyric acid	VII	10	29.8	0.132	n.a.
ϵ-amino-n-caproic acid	VIII	16	39	0.180	n.a.
Arginine	IX	21.7	17.8	1.72	n.a.
Arginine	XI	25.8	24.2	1.20	n.a.
Phenylalanine	IX	21.7	18.1	1.5	n.a.
Alanine	X	20.1	26.2	0.48	0.89
γ-amino-n-butyric acid	X	20.1	22.4	0.74	n.a.
Isoleucine	XI	25.8	23.8	1.3	n.a.
Propyl gallate	VI	17.5	44.5	0.184	n.a.

[1] In concentrations of 10^{-3} moles per mole of linoleate unless otherwise specified.

[2] Ratio of oxygen absorbed by treated samples to that absorbed by the control, at a time when the oxidation degree of the control is 10 mM O_2/M linoleate.

[3] Data reported by Marcuse (1961) in which amino acids were added to linoleate in solution. The solution was 0.04 molar in linoleate, and 2×10^{-4} molar in amino acid.

[4]A preparative run in which histidine was added in concentration of 9×10^{-3} M/M linoleate.

[5]Concentration of cysteine was 2×10^{-3} M/M linoleate.

TABLE 14. Summary of studies with histidine-C^{14}.

Study*	Oxidation level, M O_2/M linoleate	M histidine/M linoleate
A	0.2	10^{-3}
B	0.2	9×10^{-3}
C	0.075	9×10^{-3}

*The oxidation rates for studies B and C are shown in Figure 25. The run in which samples B and C were prepared is identified as Run Z.

TABLE 15. Distribution of radioactivity from histidine in study A.

Fraction*	Percentage of Total
I	2.5
II	3.0
III	94.5

*See text for description of fractions.

KEY TO TABLES 16-18

fl = Fluorescent

P+ = Positive test for peroxide

N+ = Positive test with ninhydrin

S+ = Positive test with diazotized sulfanilic acid

Rad= Radioactivity from histidine

R_H = Migration on paper chromatography relative to histidine

M_H = Migration on paper electrophoresis relative to histidine

TABLE 16. Analysis of Fraction I from Study A.

Treatment	Observations
I. Chromatography	
1. Silica Gel G (Merck)*	
a) chloroform	fl, P+, Rad--0 to 2 cm
	fl--10 cm
b) n-propanol-water (64:36)	fl, P+, Rad--5 cm
	fl, P+--7 cm
c) dioxane-water (9:1)	fl, P+, N+, Rad--0 to 2 cm
	fl, P+, N+, S+, Rad--8.5 cm
II. Electrophoresis**	
1. 5N Acetic acid (pH 2)	fl--M_H = 0
2. Borate buffer (pH 9)	fl--M_H = 0

* Maximum migration is 10 cm.

**M_H histidine = 100.

TABLE 17. Analysis of Fraction II from Study A.

Treatment	Observations
I. Chromatography	
1. Silica Gel G (Merck)*	
a) chloroform	N+--0 cm, fl--0 to 2 cm
b) n-propanol-water (64:36)	N+--0 cm, fl--2.5, 5, 7 cm
2. Paper**	
a) n-butanol-acetic acid-water (25:6:25, upper phase, descending)	fl--R_H = 0 N+, S+, Rad--R_H = 100
b) isopropanol-ammonia-water (160:8:16, ascending)	N+, S+, Rad--R_H = 100
II. Electrophoresis***	
1. Potassium hydrogen phthalate, pH 4	N+, Rad--M_H = 20 N+, Rad--M_H = 100

*Maximum migration is 10 cm.

**R_H Histidine = 100.

***M_H Histidine = 100.

TABLE 18. Analysis of Fraction III from Study A.

Treatment	Observations
I. Chromatography	
1. Paper	
a) n-butanol-acetic acid-water (25:6:25, upper phase, descending)	N+, S+, Rad--R_H = 100
	N+, S+, Rad--R_H = 250
b) isopropanol-ammonia-water (160:8:16, ascending)	streak--N+, S+, Rad from R_H = 0 to R_H = 100 (Histidine gives discreet spot)
c) methylethylketone-sec-butanol-tert-butanol-water (8:4:4:5 containing 0.5% diethylamine, ascending)	N+, S+, Rad--R_H = 100
d) ethanol-ammonia-water (180:10:10, ascending)	N+, S+, Rad--R_H = 100
II. Electrophoresis	
1. Potassium hydrogen phthalate pH 4	N+, S+, Rad--M_H = 19
	N+, S+, Rad--M_H = 100
2. Sodium carbonate, pH 11.5	N+, S+, Rad--M_H = 100
	streak--Rad--M_H = 0 to M_H = 100

TABLE 19. Two-Dimensional Chromatography and Electrophoresis of Fraction III from Study A.

Position, cm from Origin*		Percent of Total C^{14} in Fraction III
X	Y	
1	1.5	4.8
5	1.5	60.0
1	4.5	1.6
5	4.5	33.6**
		100.0

*Electrophoresis in the X-direction in phthalate buffer (pH 4), chromatography in the Y-direction in n-butanol-acetic acid-water (25:6:25, upper phase, descending).

**The coordinates for histidine in this system are X--5 cm, Y--4.5 cm.

TABLE 20. Column chromatography of systems* containing methyl linoleate-U-C^{14}.

Eluting Solvent	% of Total Radioactivity		
	With Histidine, Unoxidized	With Histidine, Oxidized	With No Histidine Oxidized
Petroleum ether	93.80	94.30	93.40
Chloroform-methanol (4:1)	6.00	5.45	6.37
Chloroform-methanol (3:1)	0.06	0.12	0.07
Chloroform-methanol (2:1)	0.05	0.06	0.03
Chloroform-methanol (1:1)	0.06	0.04	0.07
Water	0.03	0.03	0.06
	100.00	100.00	100.00

*Freeze-dried system with or without histidine as designated. All systems contained 10^{-3} M/ histidine/M linoleate; oxidized systems contained 0.075 M O_2/M linoleate.

TABLE 21. Thin layer chromatography of methionine reaction products on silica gel.

Sample	R_f in Solvent System	
	A	B
Methionine	0.50	0.33
Methionine sulfoxide	0.25	0.10
Methionine sulfone	0.35	0.20
Oxidized system containing linoleate	0.50, 0.25	0.33, 0.10
Oxidized system without linoleate	0.50	0.33

Solvent systems:

A = n-propanol-water (2:1)

B = sec-butanol-acetic acid-water (4:1:1)

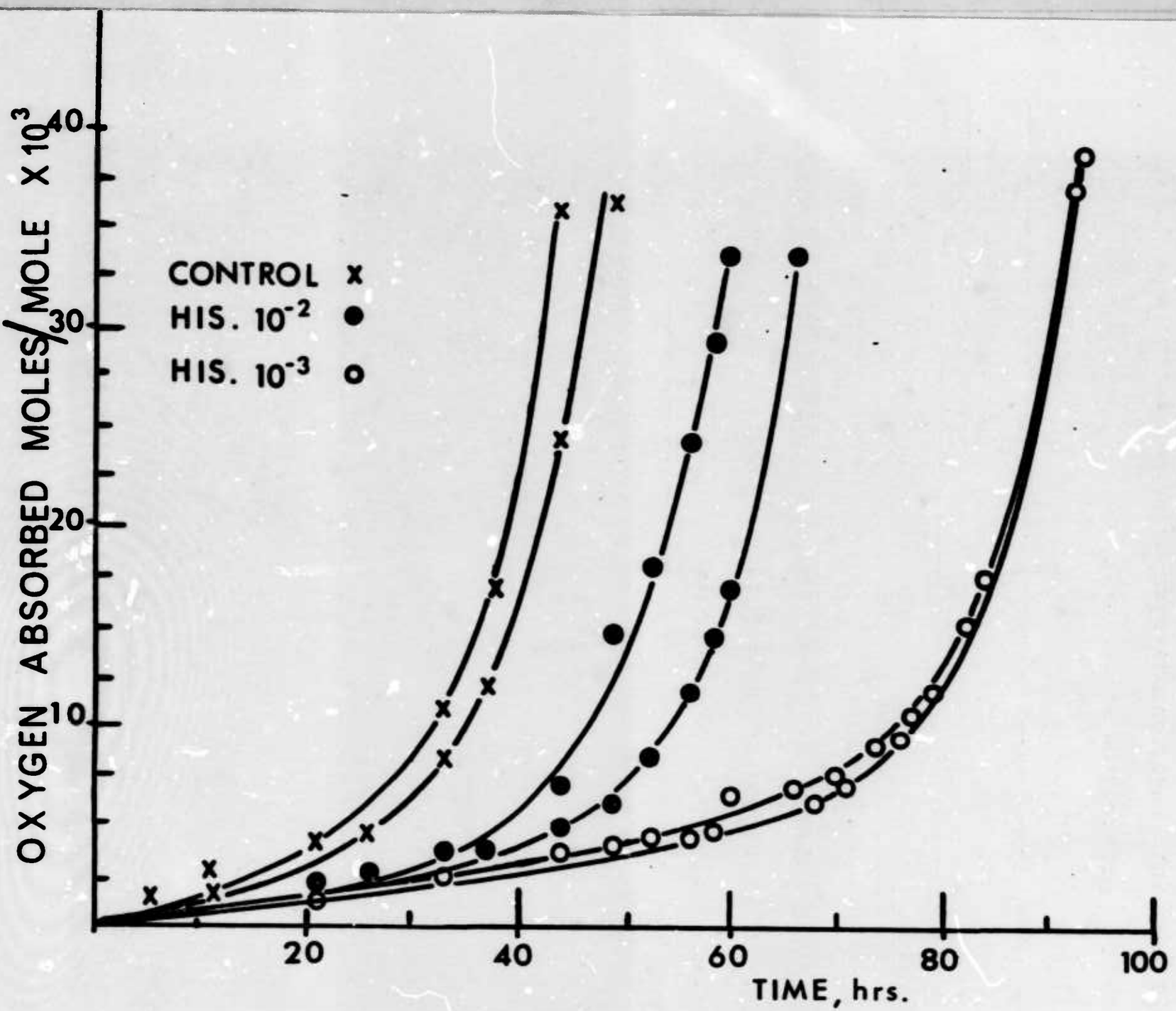

Figure 1. Oxygen absorbed by model system in initial stages of oxidation. Run I. (Additive: histidine)

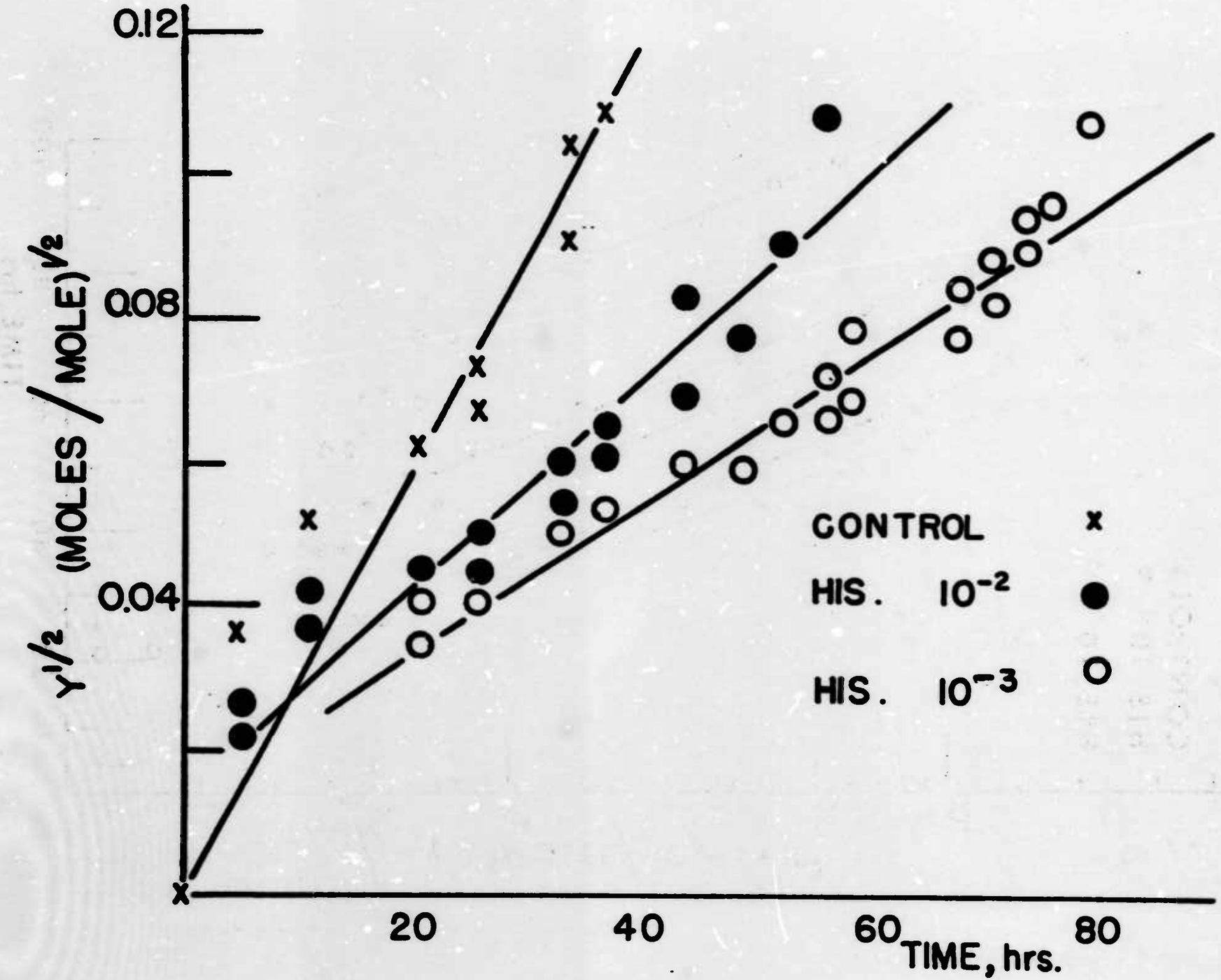

Figure 2. Plot for monomolecular decomposition period. Run I. (Additive: histidine)

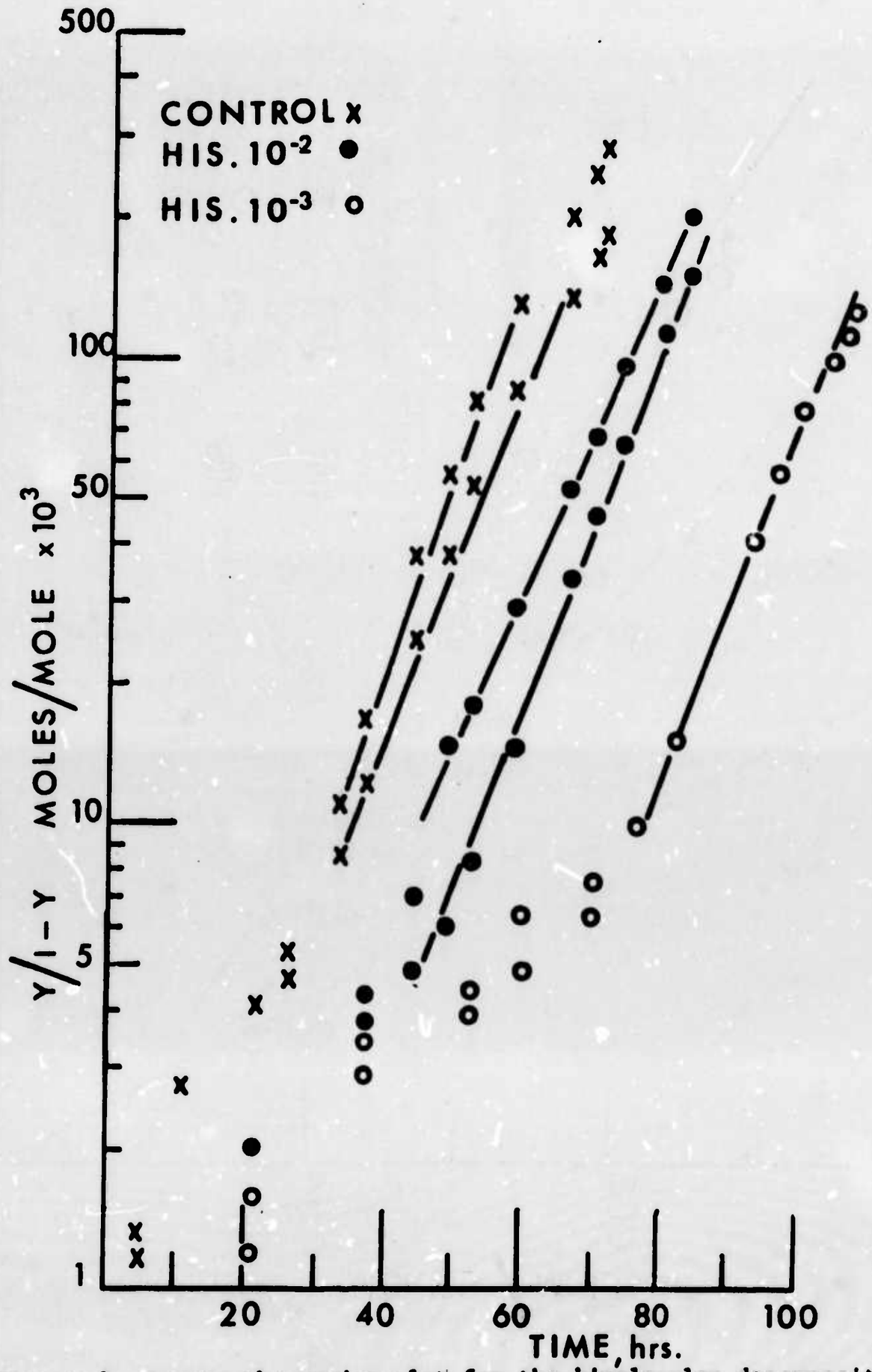

Figure 3. Oxygen absorption plot for the bimolecular decomposition. Run I. (Additive: histidine)

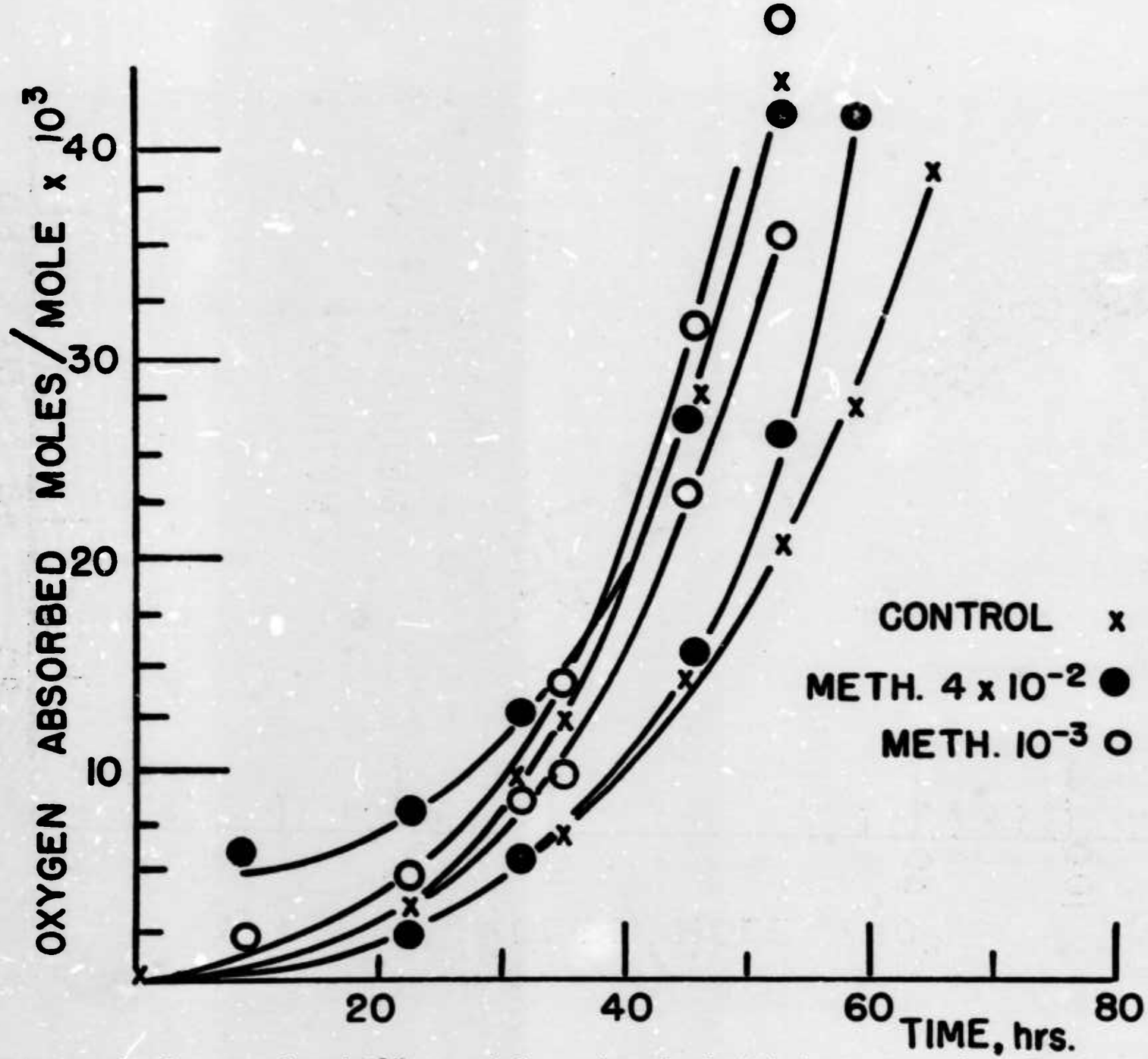

Figure 4. Oxygen absorbed by model system in initial stages of oxidation. Run II (Additive: methionine)

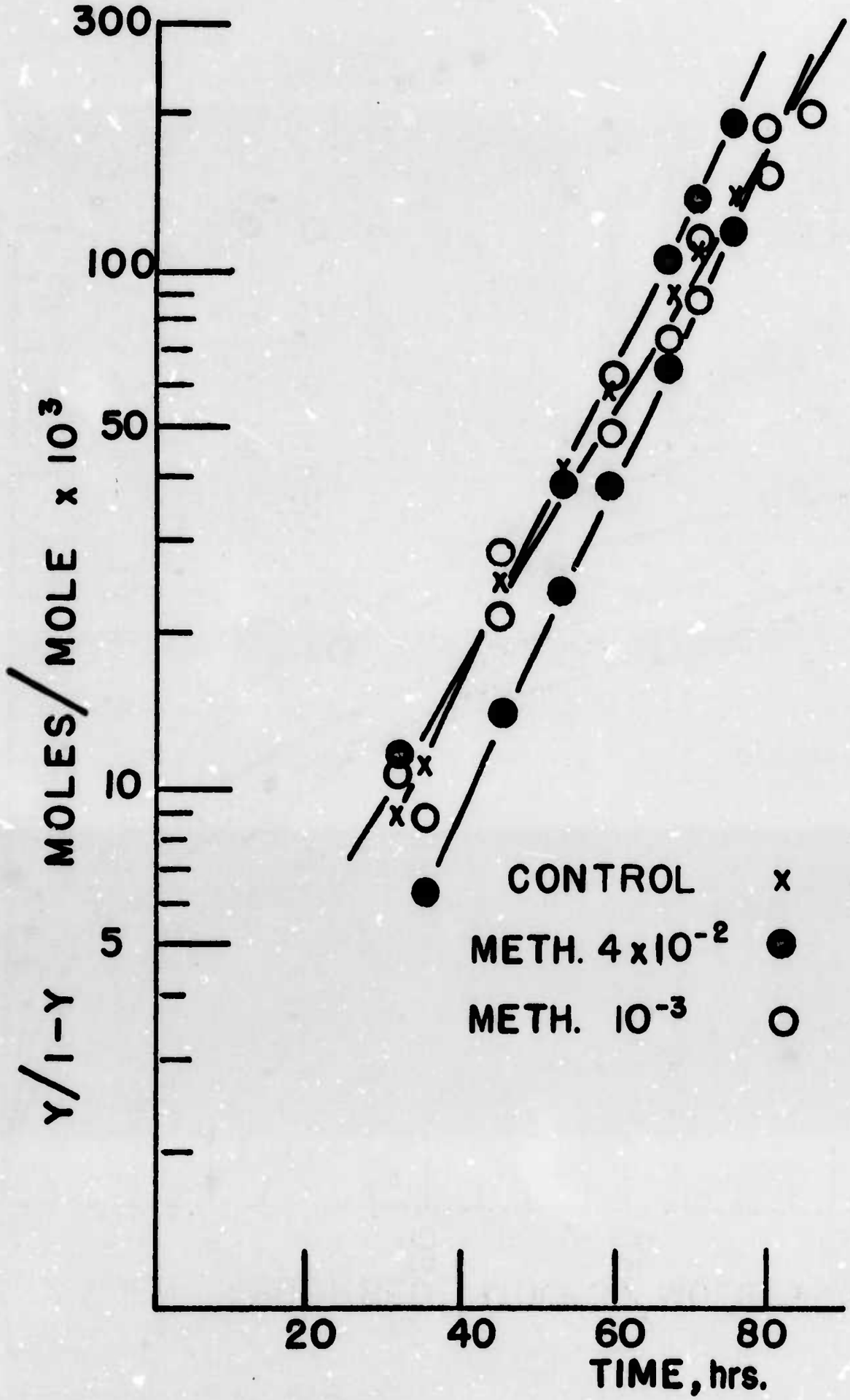

Figure 5. Oxygen absorption plot for the bimolecular decomposition. Run II (Additive: methionine)

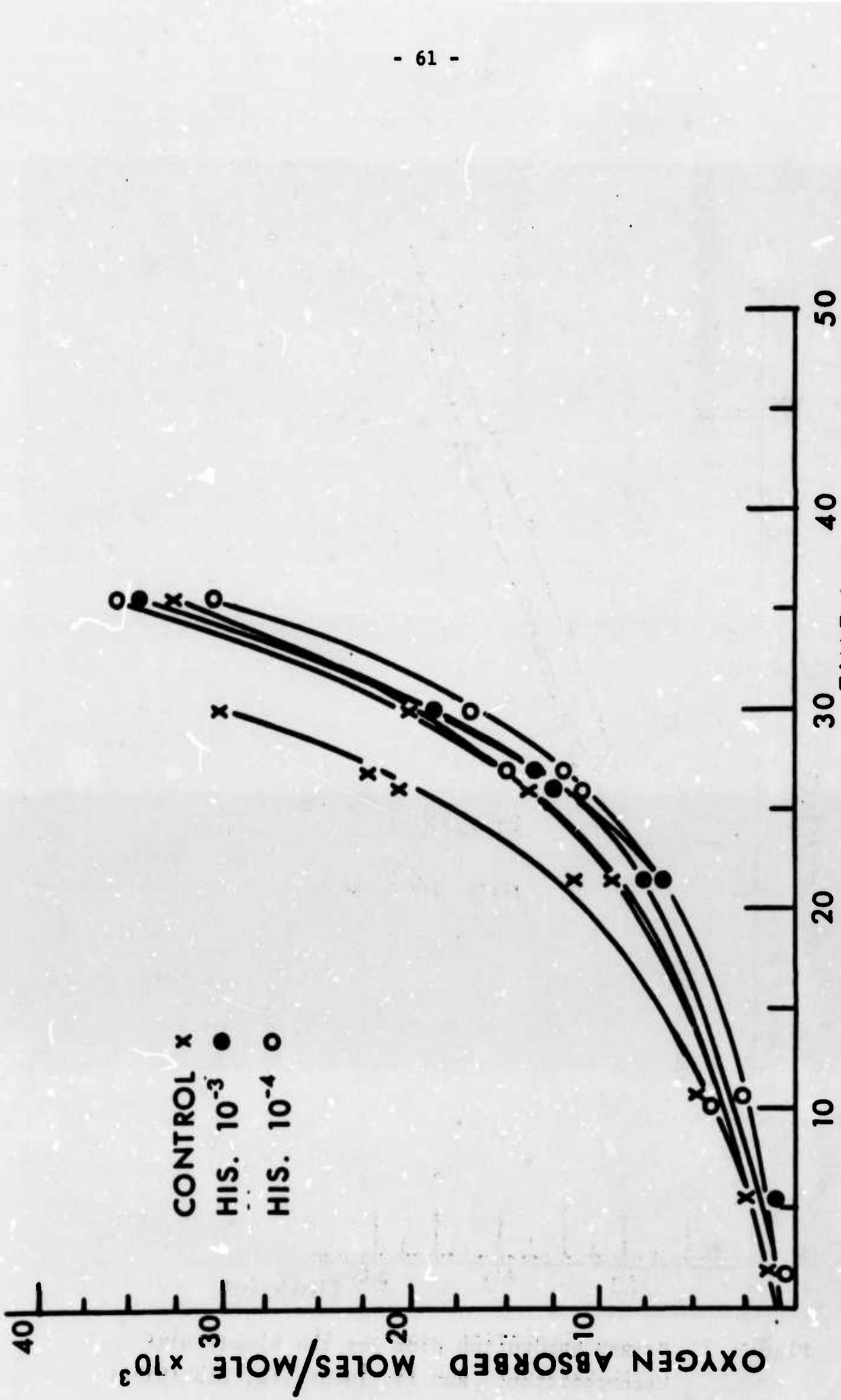

Figure 6. Oxygen absorbed by model system in initial stages of oxidation. Run IV. (Additive: histidine)

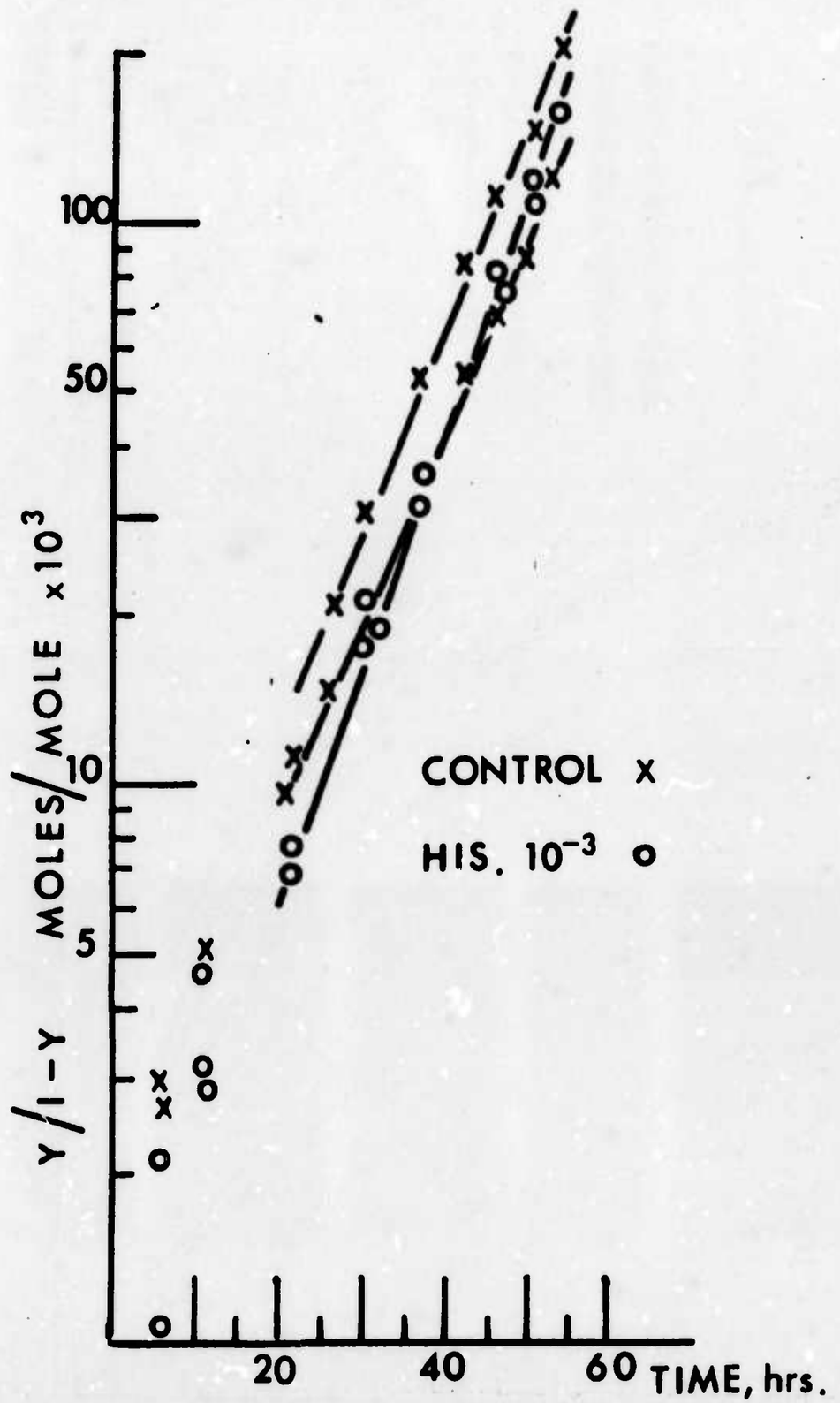

Figure 7. Oxygen absorption plot for the bimolecular decomposition. Run IV. (Additive: histidine)

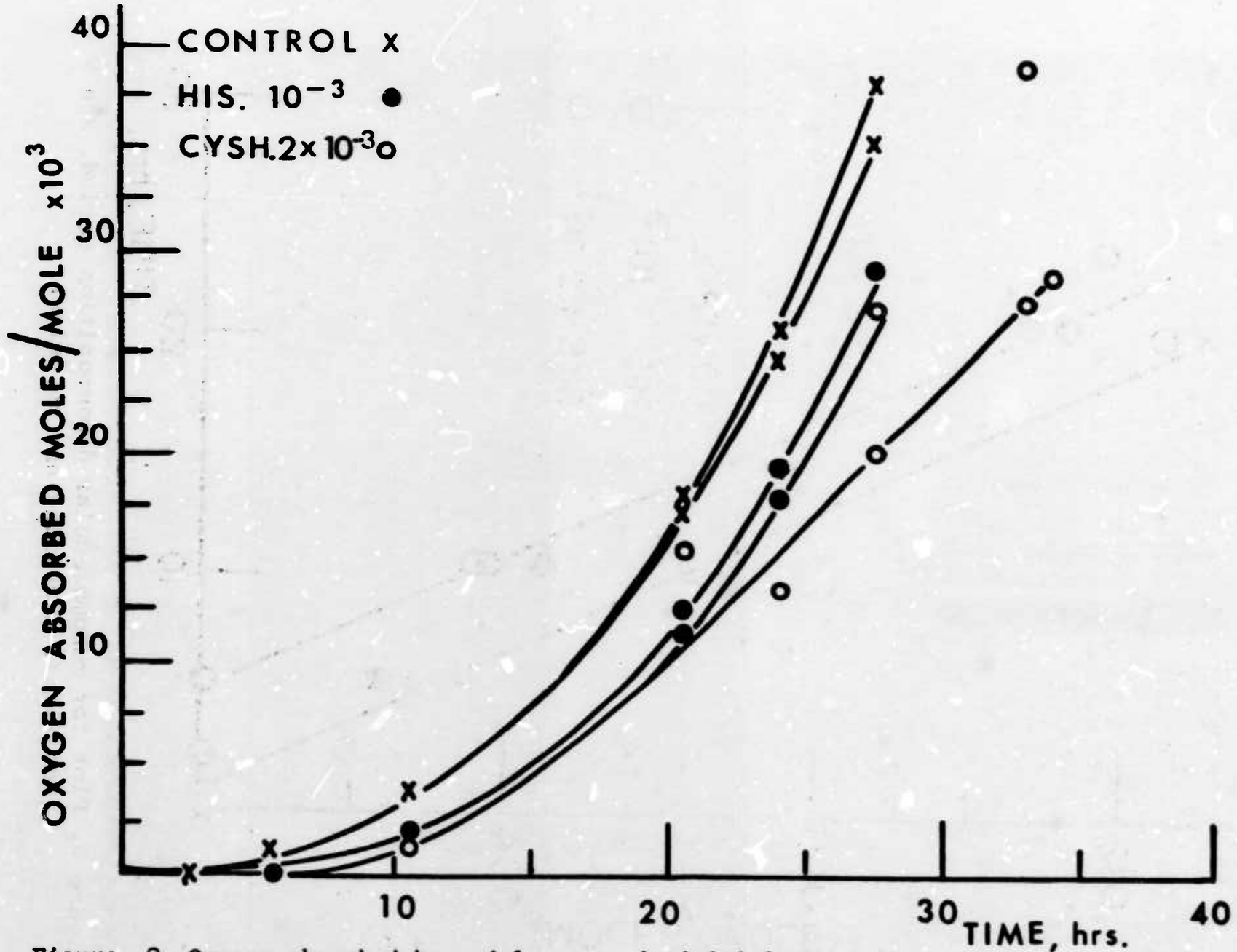

Figure 8. Oxygen absorbed by model system in initial stages of oxidation. Run V. (Additives: histidine, cysteine)

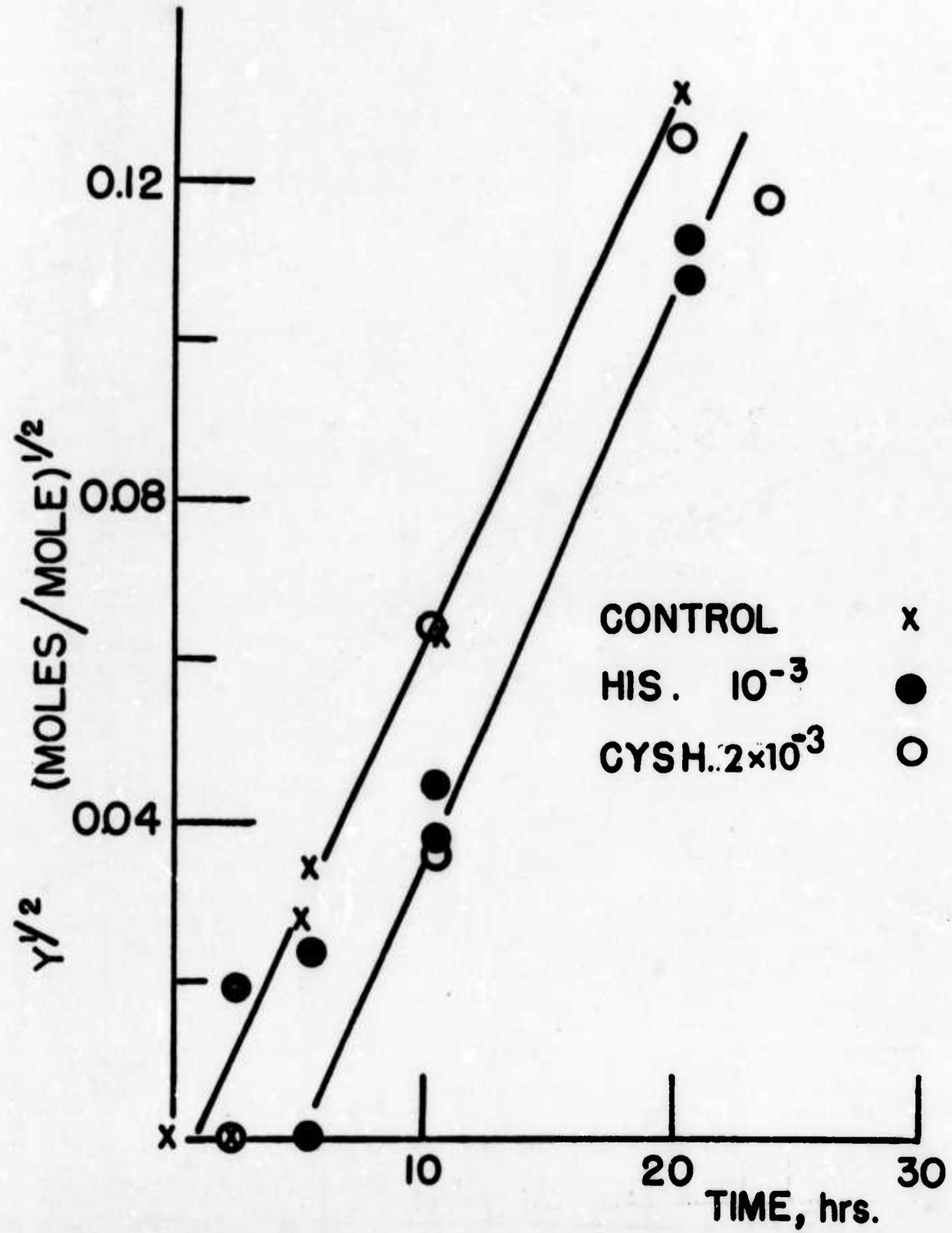

Figure 9. Plot for monomolecular decomposition period. Run V . (Additives: histidine and cysteine)

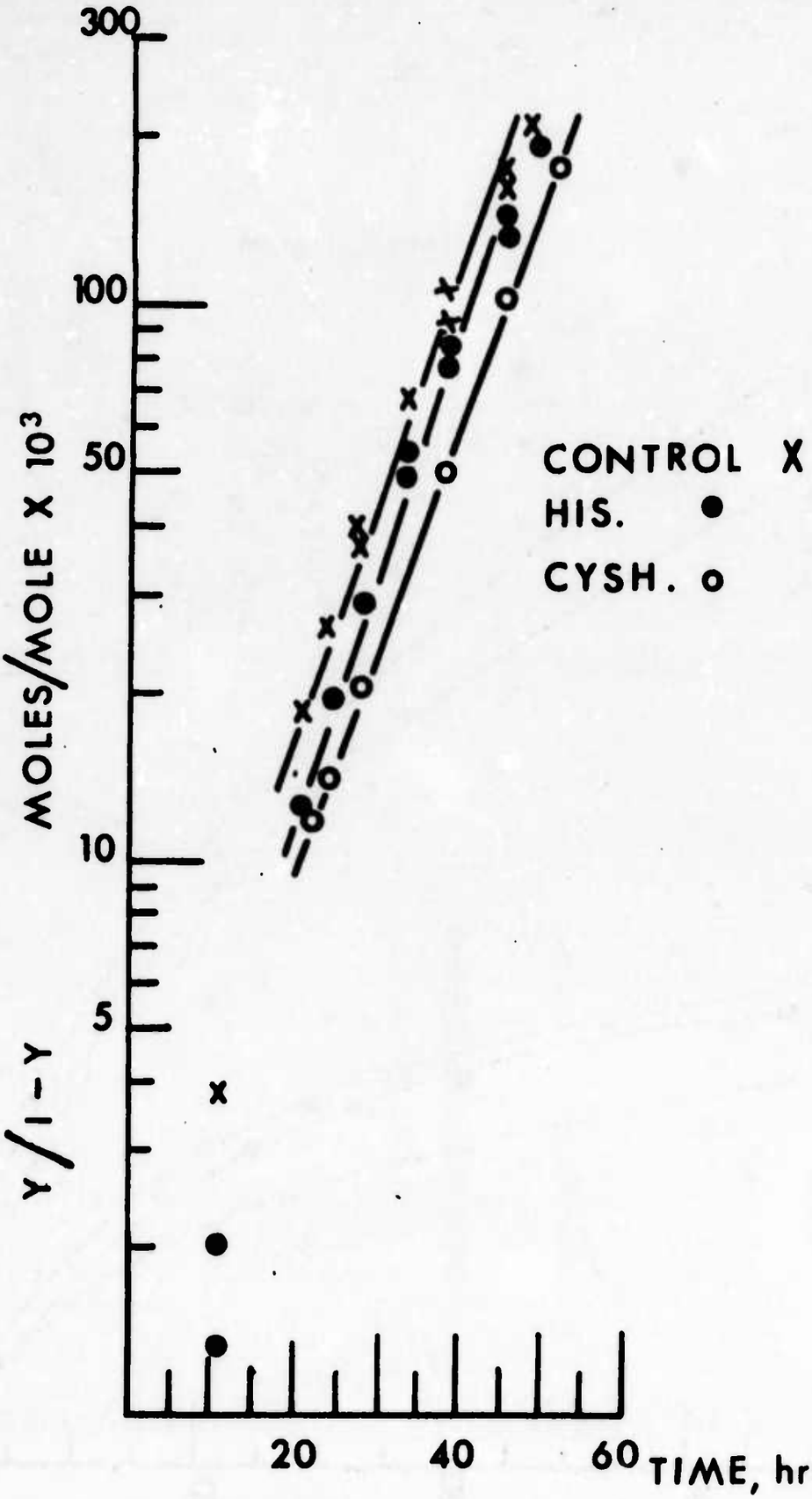

Figure 10. Oxygen absorption plot for the bimolecular decomposition. Run V. (Additives: histidine, cysteine)

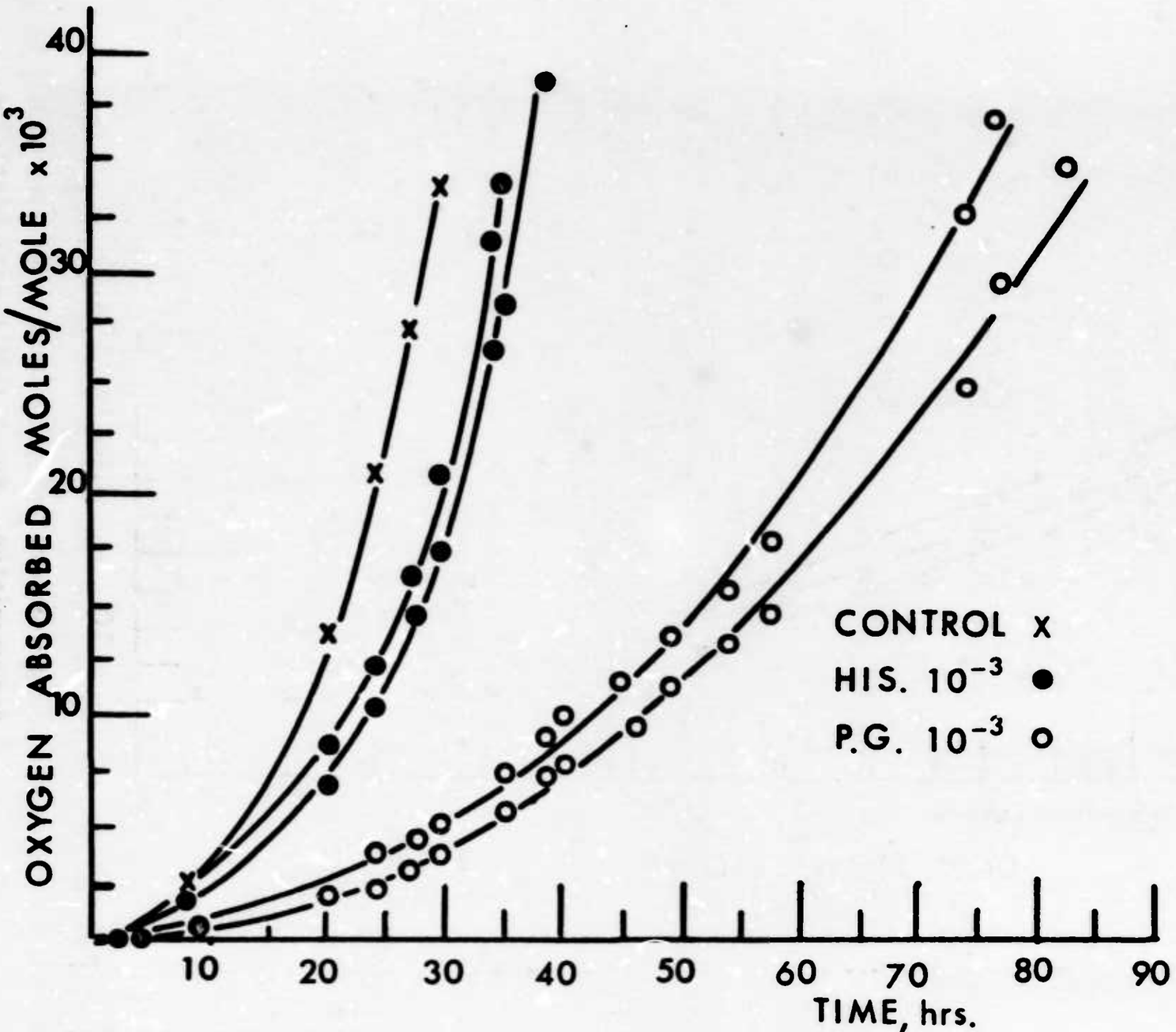

Figure 11. Oxygen absorbed by model system in initial stages of oxidation. Run VI. (Additives: histidine, propyl gallate)

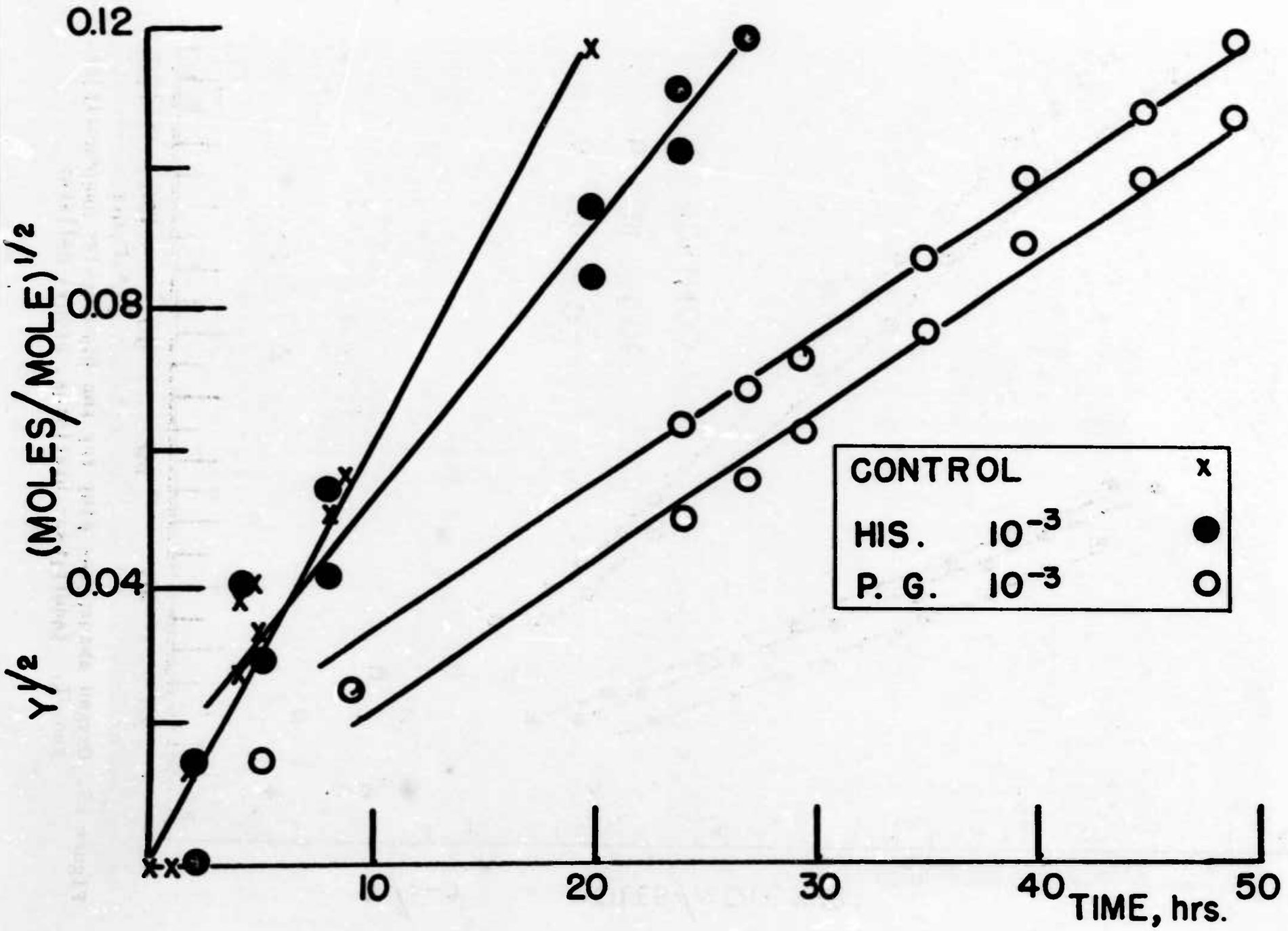

Figure 12. Plot for monomolecular decomposition period. Run VI (Additives: histidine and propyl gallate)

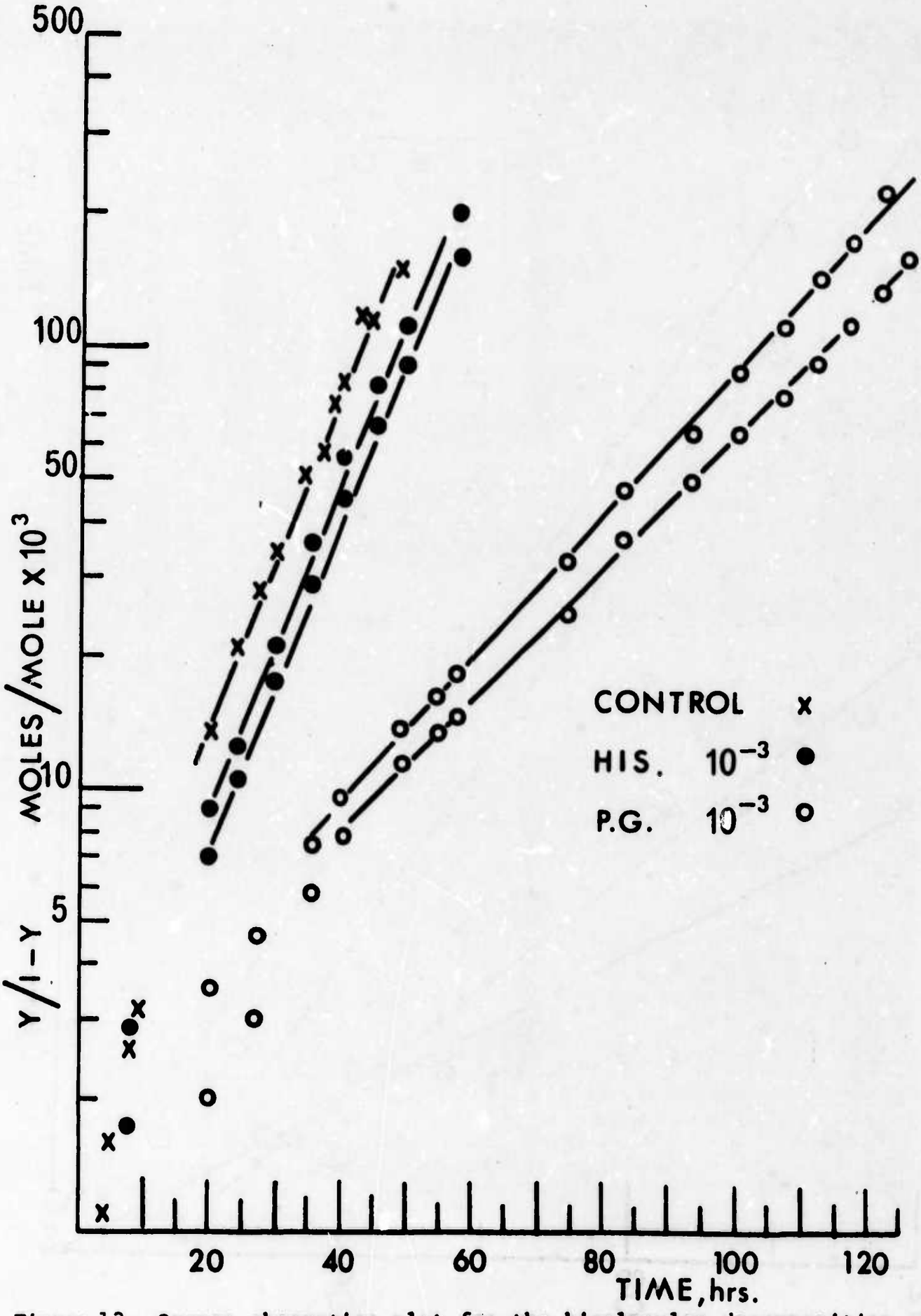

Figure 13. Oxygen absorption plot for the bimolecular decomposition. Run VI. (Additives: histidine, propyl gallate)

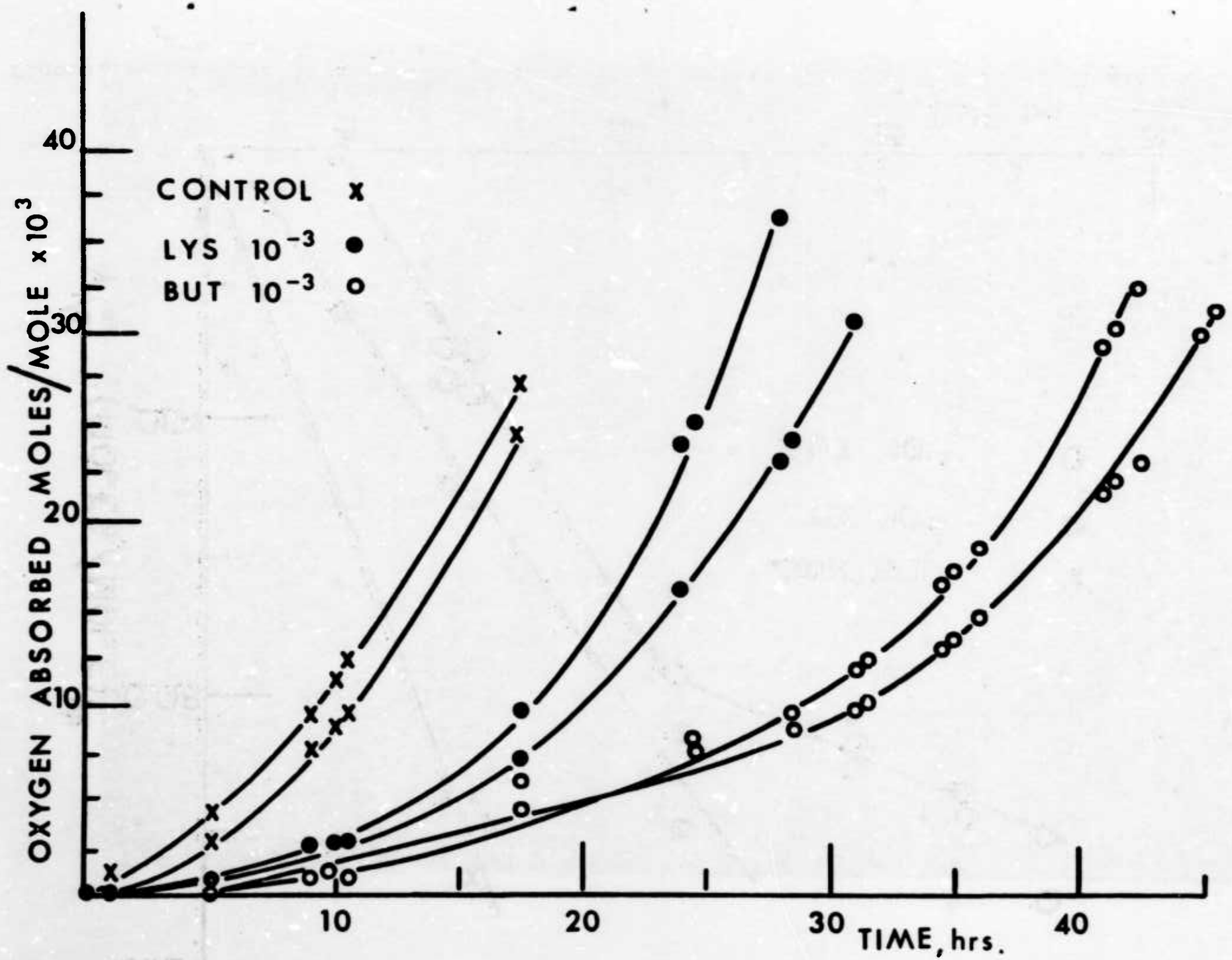

Figure 14. Oxygen absorbed by model system in initial stages of oxidation. Run VII. (Additives: lysine, β-amino-n-butyric acid)

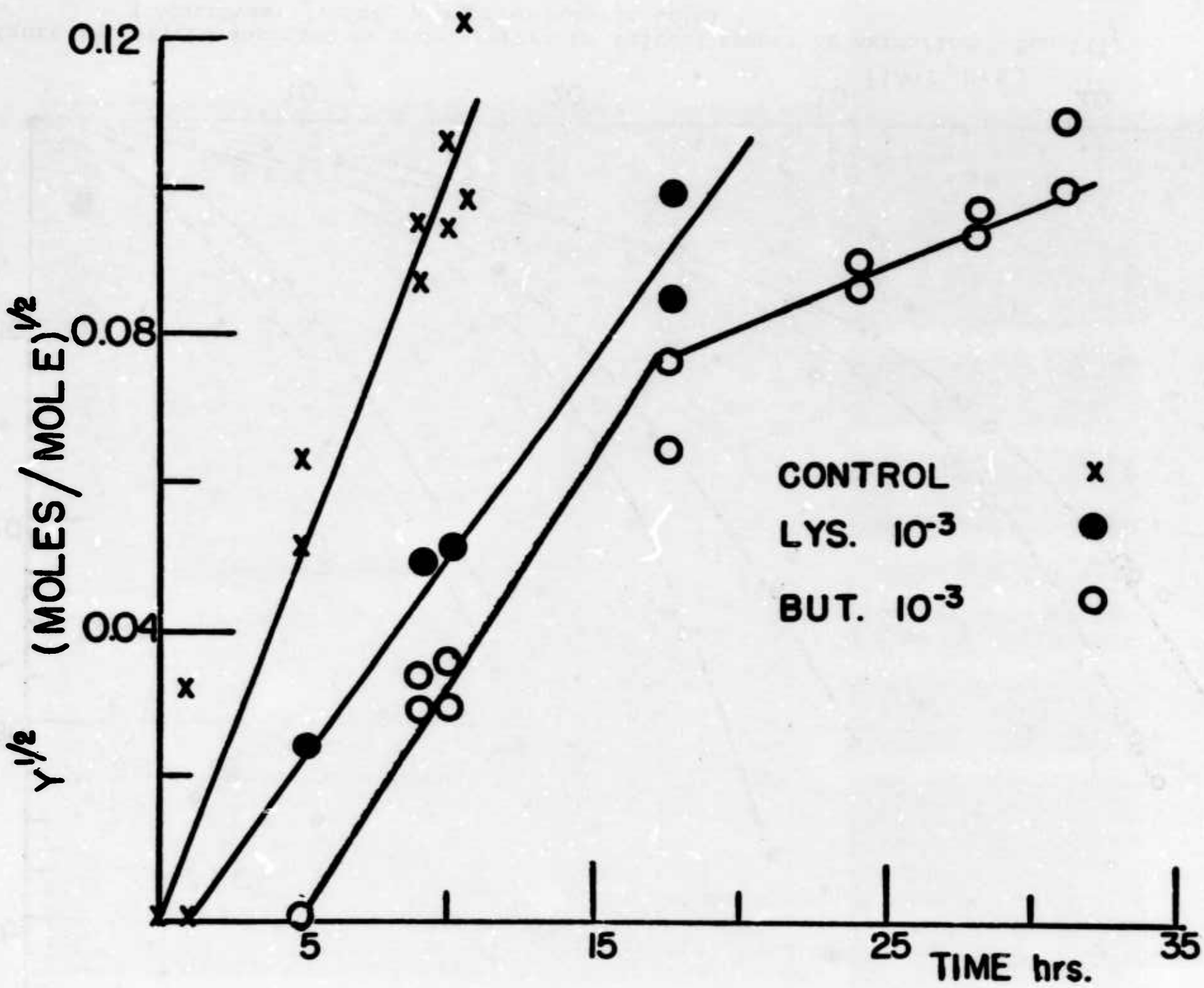

Figure 15. Plot for monomolecular decomposition period. Run VII (Additives: lysine and β-amino-n-butyric acid)

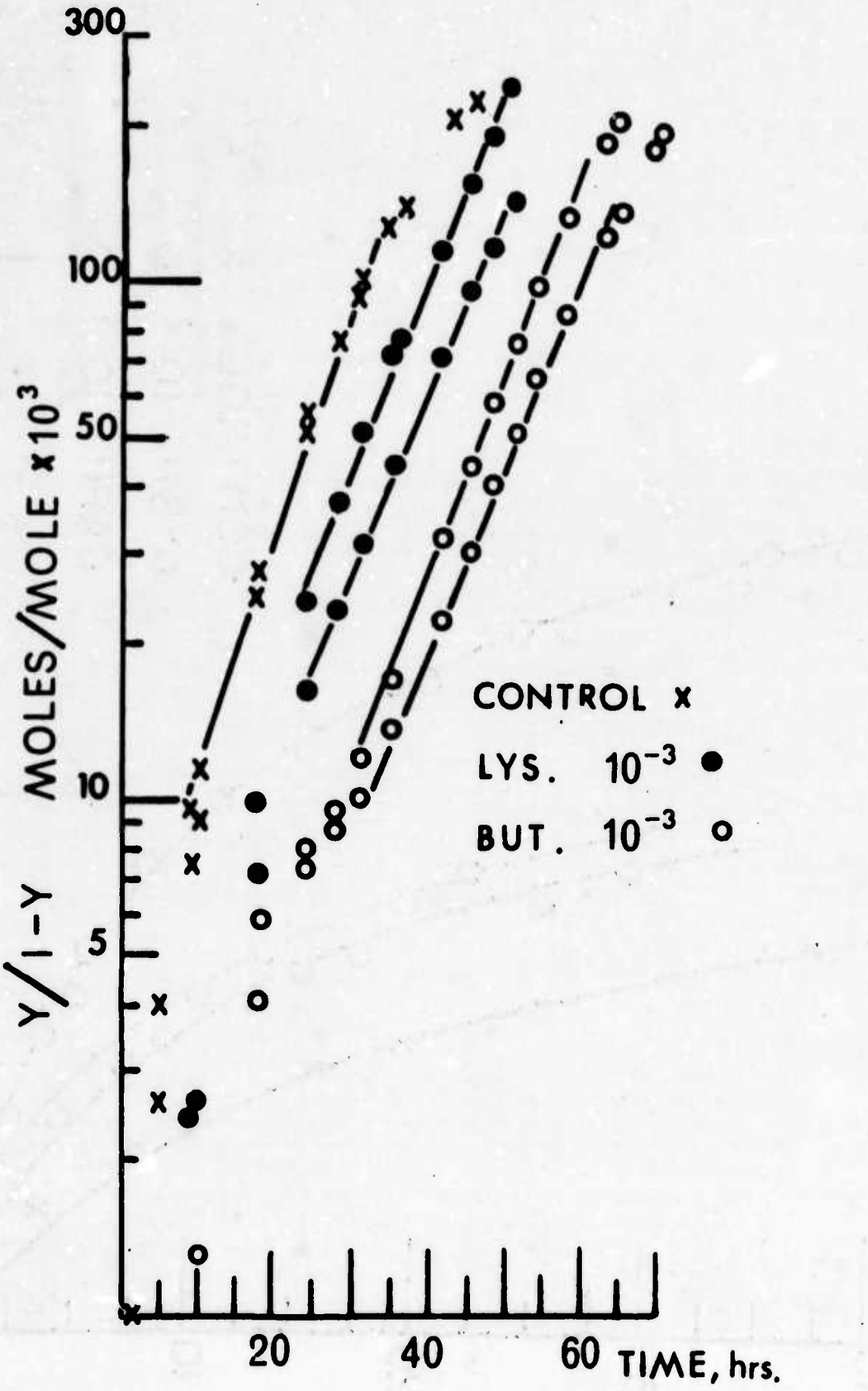

Figure 16. Oxygen absorption plot for the bimolecular decomposition. Run VII. (Additives: lysine, β-amino-n-butyric acid)

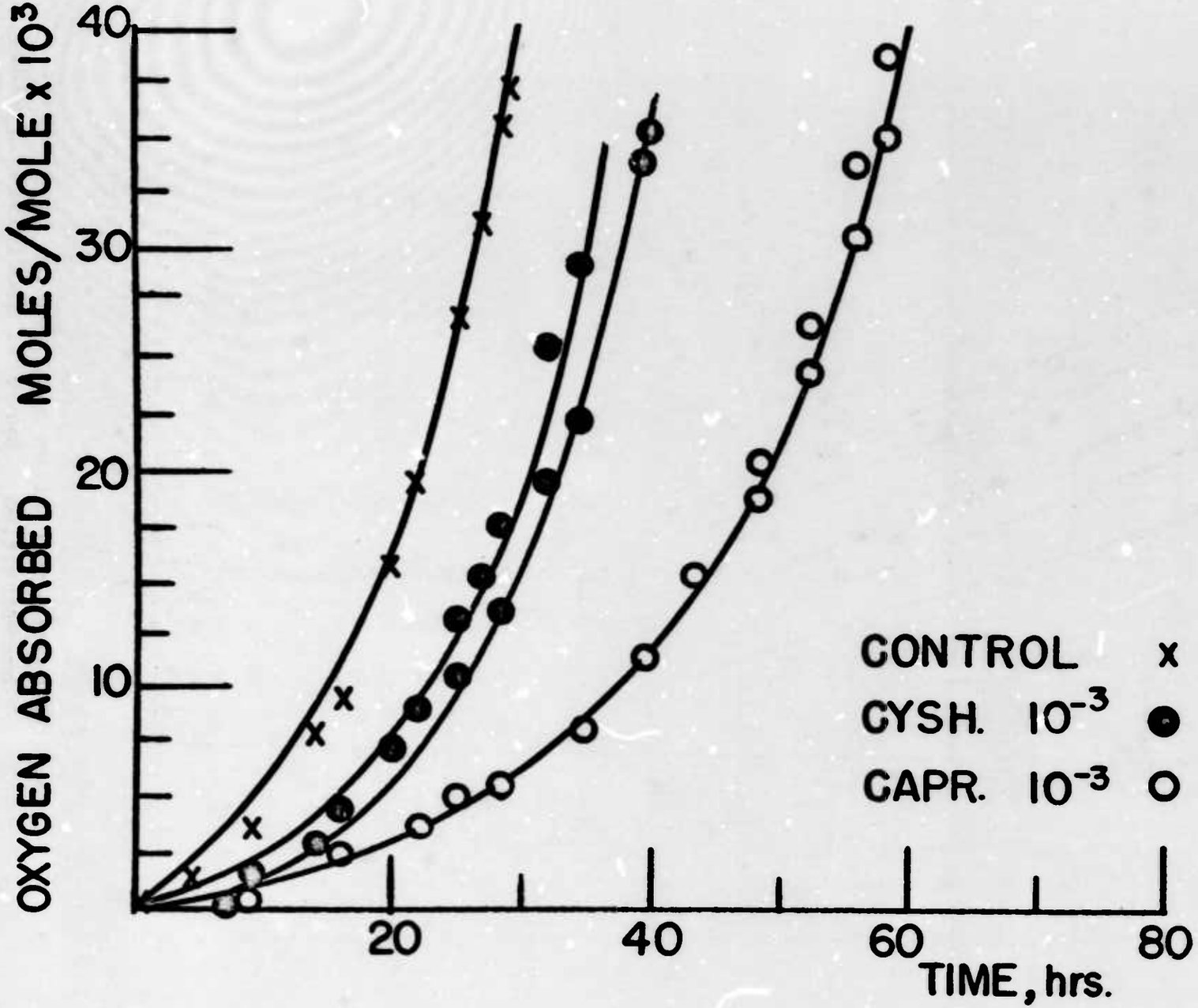

Figure 17. Oxygen absorbed by model system in initial stages of oxidation. Run VIII (Additives: cysteine and ε-amino-n-caproic acid)

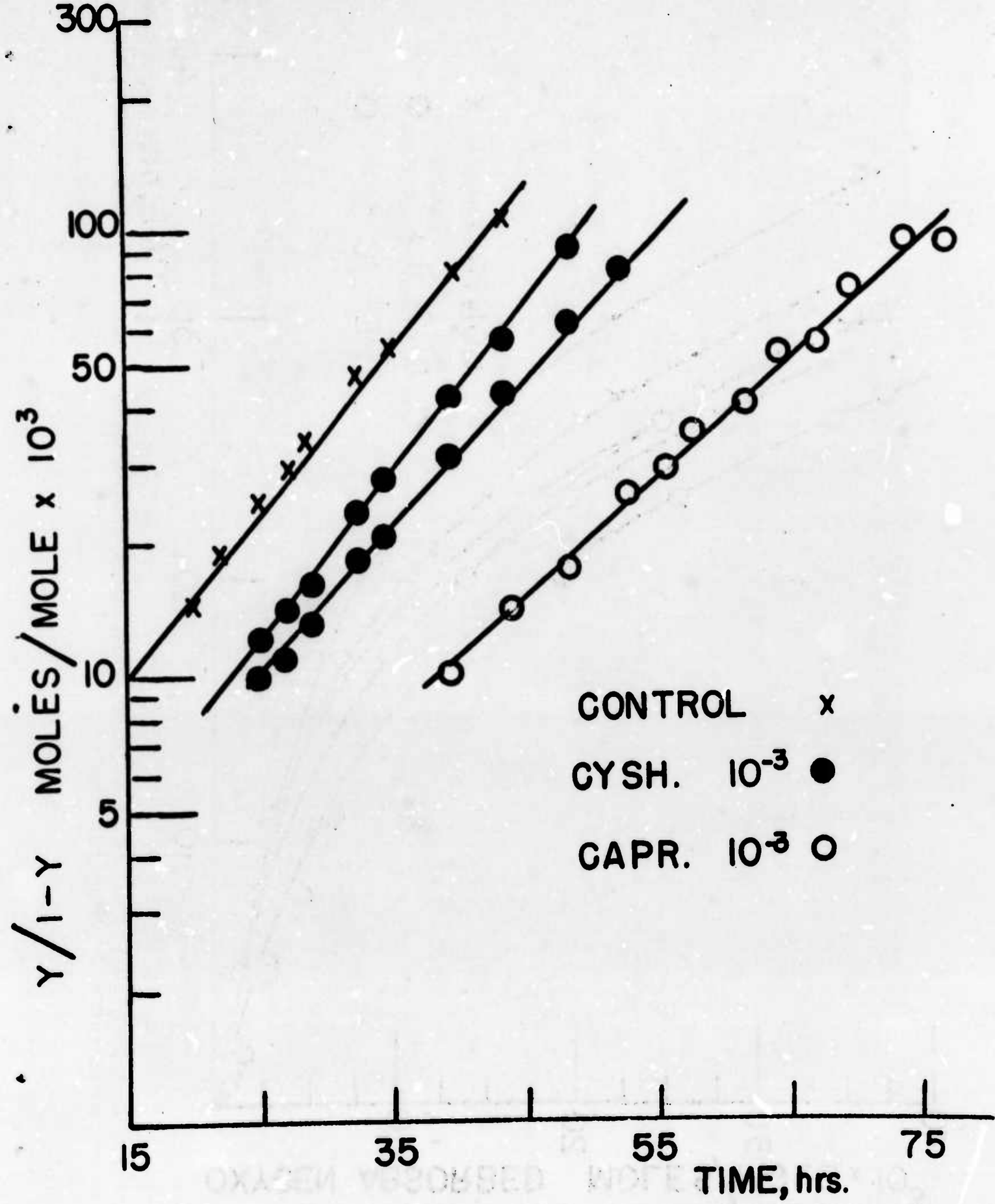

Figure 18. Oxygen absorption plot for the bimolecular decomposition. Run VII
(Additives: cysteine and ε-amino-n-caproic acid)

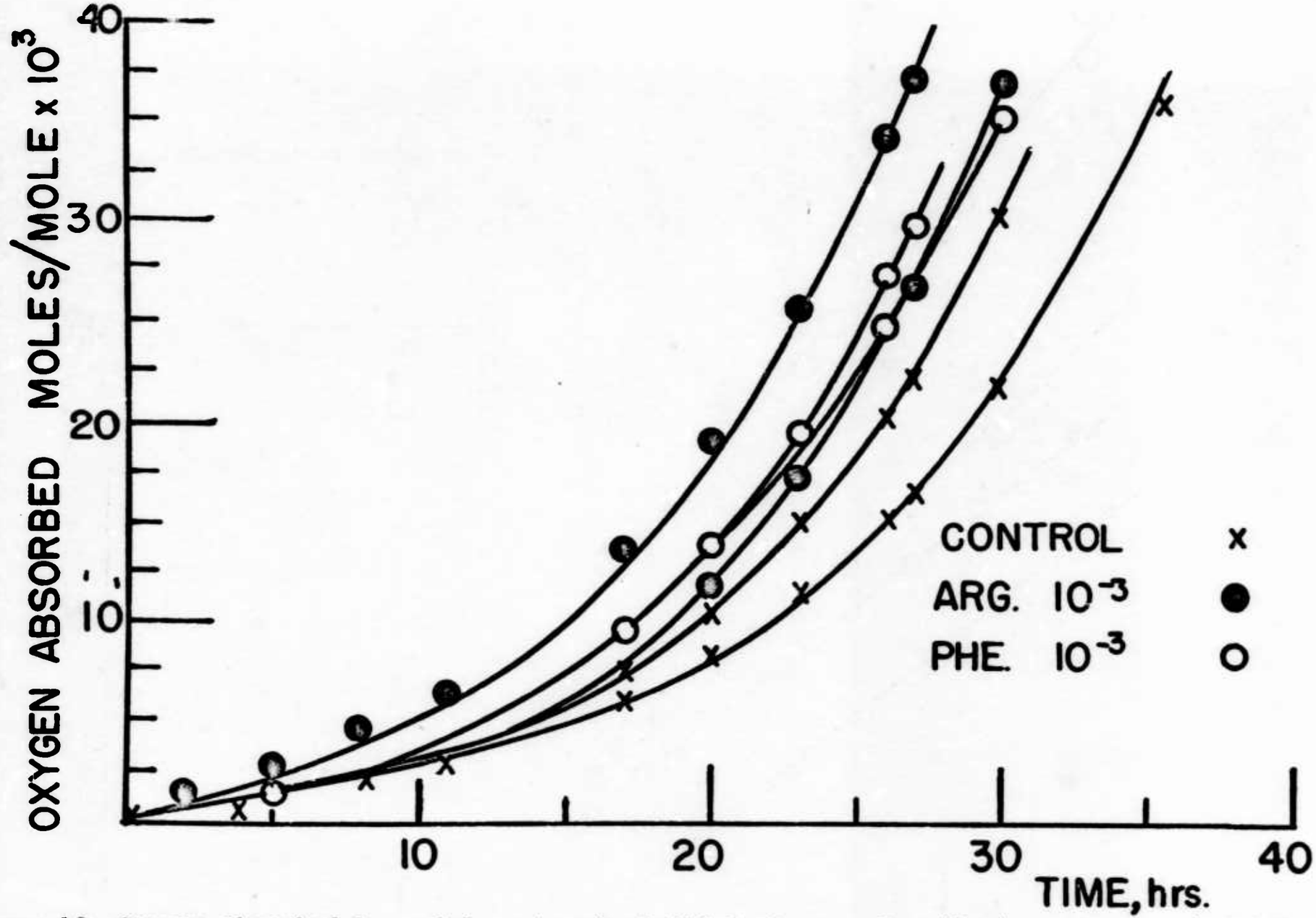

Figure 19. Oxygen absorbed by model system in initial stages of oxidation. Run IX (Additives: arginine and phenylalanine)

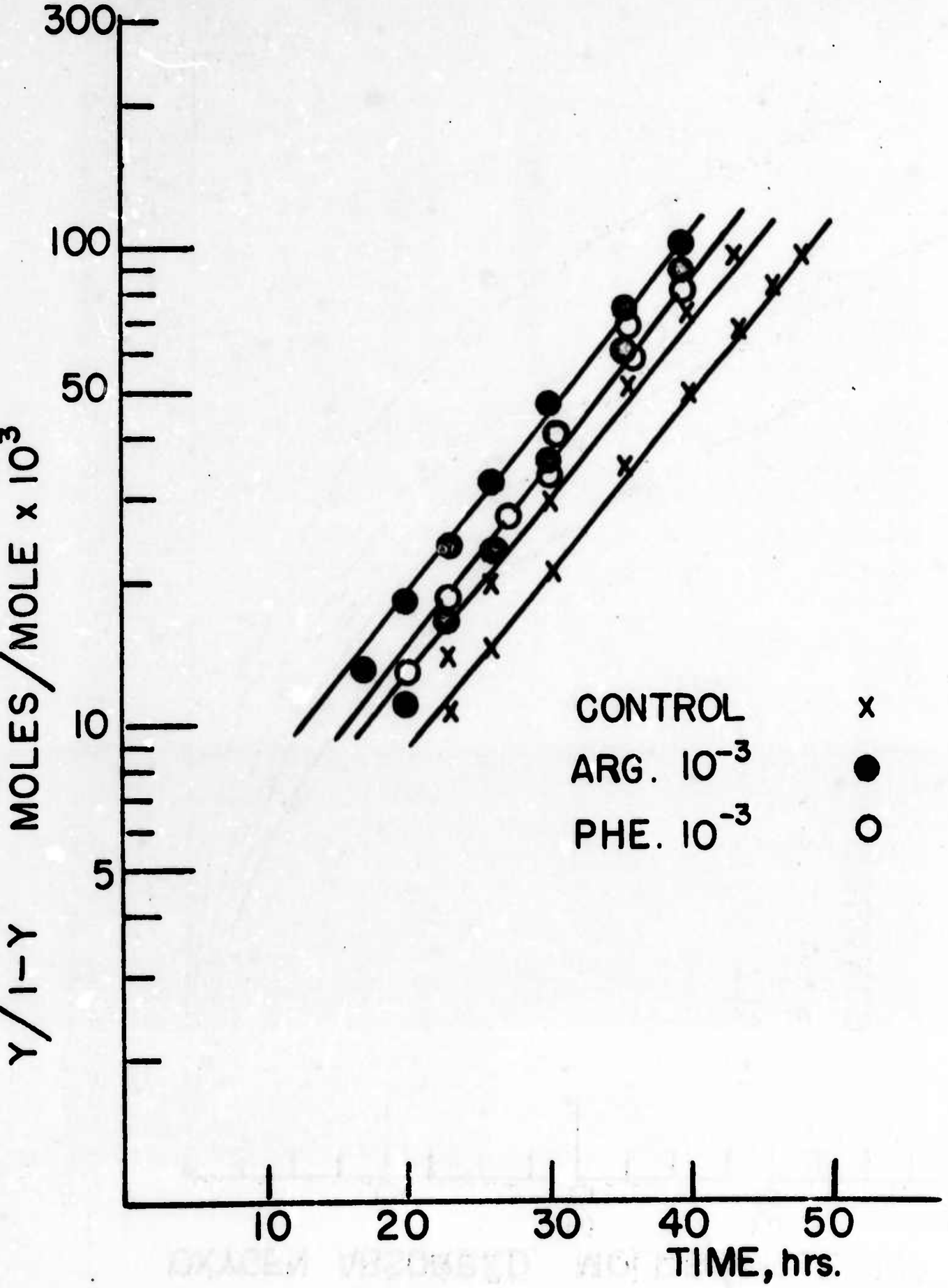

Figure 20. Oxygen absorption plot for the bimolecular decomposition. Run IX. (Additives: arginine and phenylalanine)

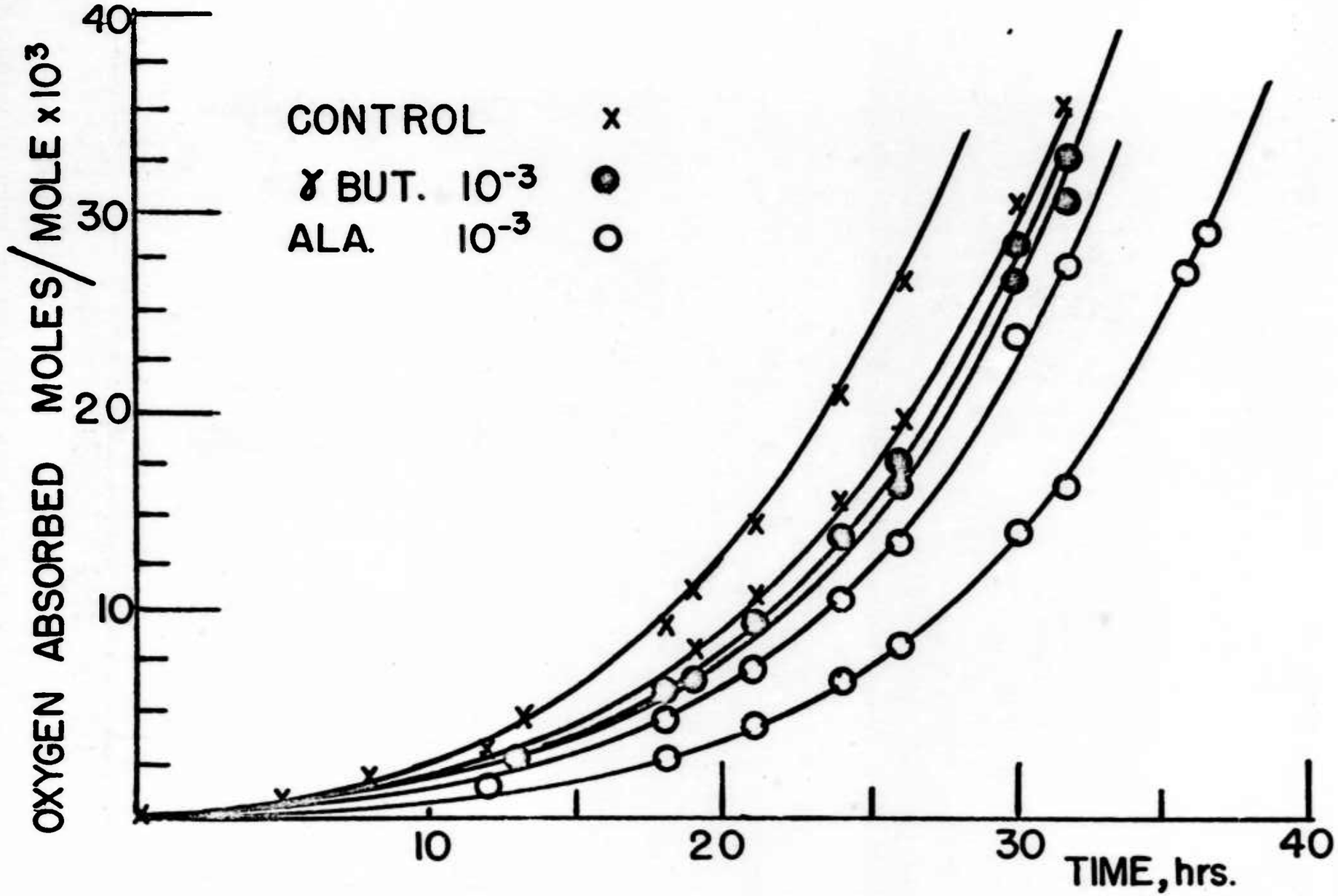

Figure 21. Oxygen absorbed by model system in initial stages of oxidation. Run X (Additives: alanine and γ-amino-n-butyric acid)

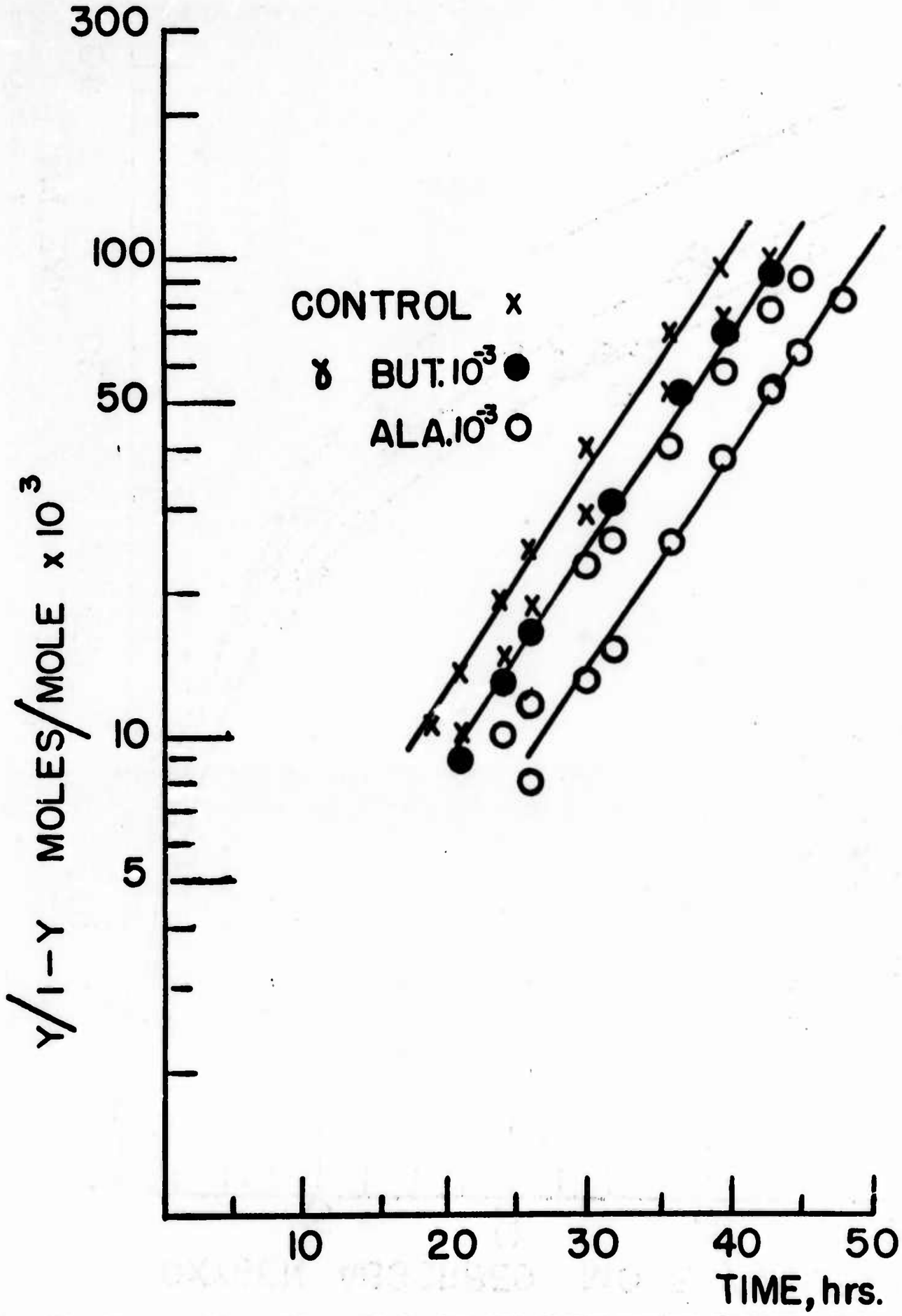

Figure 22. Oxygen absorption plot for the bimolecular decomposition. Run X
(Additives: alanine and γ-amino-n-butyric acid)

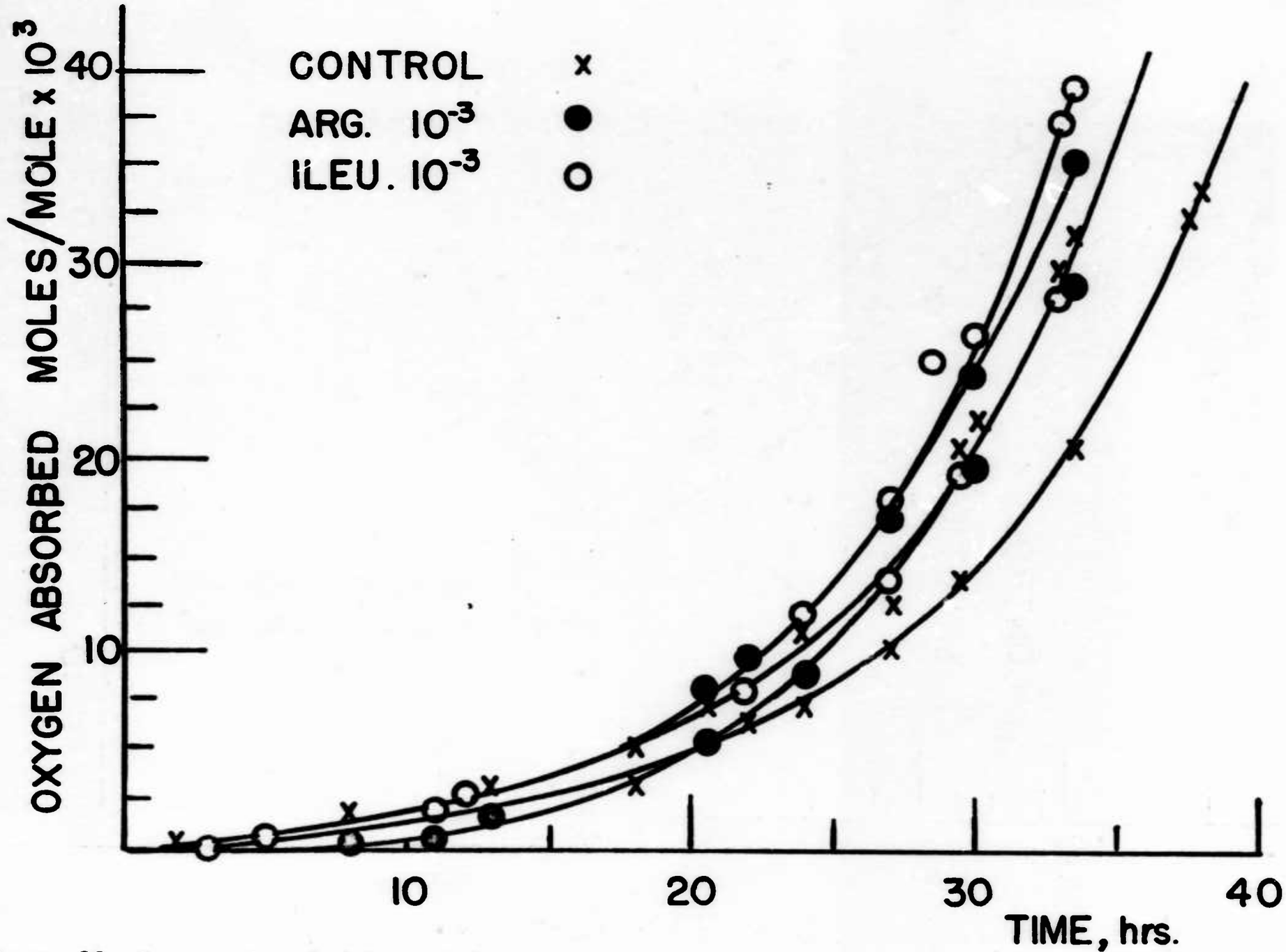

Figure 23. Oxygen absorbed by model system in initial stages of oxidation. Run XI. (Additives: arginine and isoleucine)

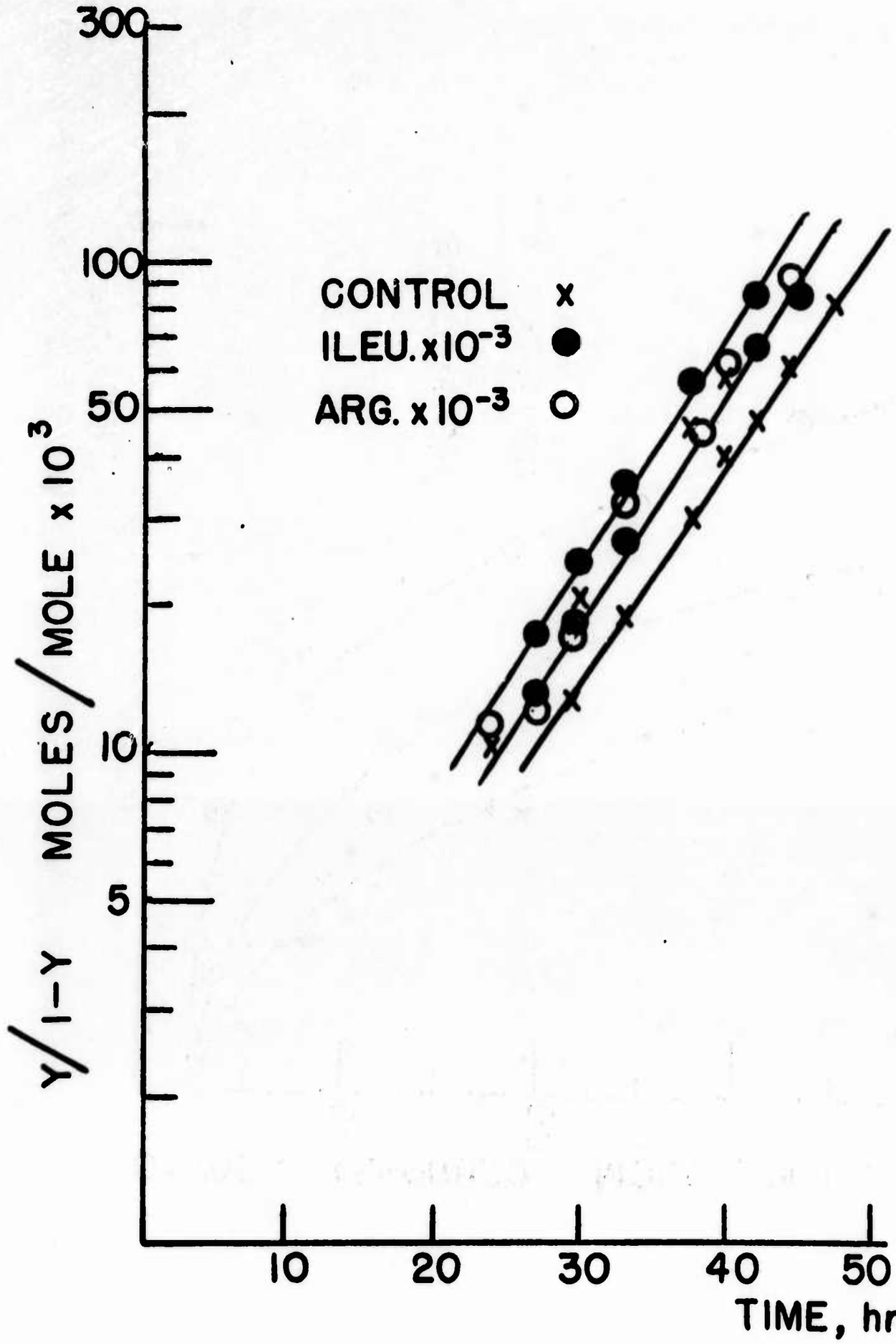

Figure 24. Oxygen absorption plot for the bimolecular decomposition Run X
(Additives: arginine and isoleucine)

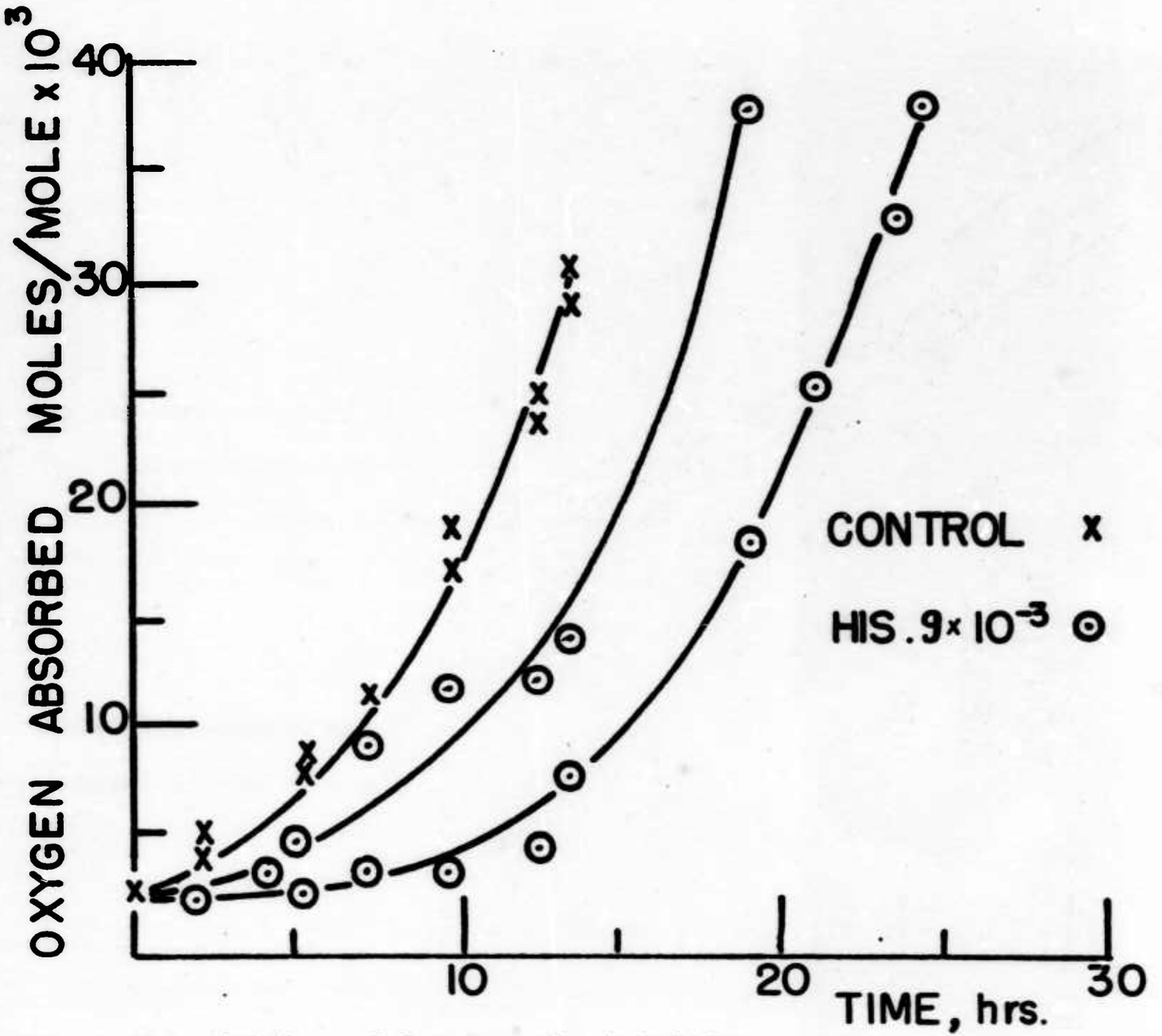

Figure 25. Oxygen absorbed by model system in initial stages of oxidation. Run Z (Additive: histidine)

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13. ABSTRACT

Oxidation of methyl linoleate was studied in a freeze-dried model system based on microcrystalline cellulose, in the presence and in the absence of amino carboxylic compounds added in concentrations ranging from 10^{-3} to 4×10^{-2} moles per mole of linoleate. It was observed that several amino carboxylic compounds had substantial antioxidant activity. Compounds for which such activity was observed included: histidine, cysteine, alanine, lysine, beta amino butyric acid, gamma amino butyric acid, and epsilon amino caproic acid; whereas no antioxidant activity was observed with methionine, arginine, phenylalanine and isoleucine.

The nature of the antioxidant activity of the amino compounds was found to be different from that observed in the identical system with a phenolic antioxidant, since the main effect of the amino compound was to prolong the induction period, but the phenolic compound prolonged the induction period, and in addition reduced the rate of oxidation throughout the course of oxidation.

Work was undertaken on the separation and characterization of reaction products from model systems containing linoleate and amino compounds. Reaction products in the model system oxidized in the presence of linoleate and histidine were studied using radioactive tracer compounds. Reaction products derived from histidine were located through the use of ring labelled histidine (C^{14}) and those derived from linoleate through the use of uniformly labelled methyl linoleate

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Oxidation	8		7			
Methyl lineolate	1		7			
Amino compounds	10,1		6			
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